

Basic Concepts in Immunology: Effector Cells and the Innate Immune Response

Highlights

- An immune response is the process for eliminating an offending stimulus. The clinical evidence of an immune response is inflammation.
- The immune system is composed of tissues, cells, and molecules that mediate response to infection or foreign material.
- Immune responses are broadly defined as innate or adaptive with close interaction between the two.
- Innate (natural) immunity provides immediate protection and requires no prior contact with the foreign substance or organism.
- Adaptive (acquired) immunity develops more slowly than innate immunity but provides more specific defense against infections.

Definitions

An immune response is a sequence of molecular and cellular events intended to rid the host of a threat: offending pathogenic organisms, toxic substances, cellular debris, or neoplastic cells. There are 2 broad categories of immune responses, *innate* and *adaptive*.

Innate immune responses, or *natural immunity*, require no prior contact with or “education” about the stimulus against which they are directed. Adaptive (or *acquired*) responses are higher-order, more specific responses directed against unique antigens. The term *antigen* refers to any substance (eg, toxin, foreign protein, bacterium) that can induce an immune response. Chapter 2 discusses adaptive responses in detail. This chapter introduces the crucial cells of the immune system and their functions in innate immunity.

Abbas AK, Lichtman AH, Pillai S. *Basic Immunology: Functions and Disorders of the Immune System*. 5th ed. Elsevier/Saunders; 2016.

Abbas AK, Lichtman AH, Pillai S. *Cellular and Molecular Immunology*. 9th ed. Elsevier/Saunders; 2018.

Murphy KM. *Janeway's Immunobiology*. 8th ed. Garland Science; 2012.

Cellular Components of the Immune System

White blood cells, or *leukocytes*, include several kinds of nucleated cells that can be distinguished by the shape of their nuclei and the presence or absence of cytoplasmic granules, as well as by their uptake of various histologic stains (Fig 1-1). They can be broadly divided into 2 subsets:

- myeloid (neutrophils, eosinophils, basophils and mast cells, monocytes and macrophages, and dendritic cells and Langerhans cells)
- lymphoid (T lymphocytes, B lymphocytes, and natural killer cells)

See BCSC Section 4, *Ophthalmic Pathology and Intraocular Tumors*, for additional discussion of leukocytes and findings on histologic examinations.

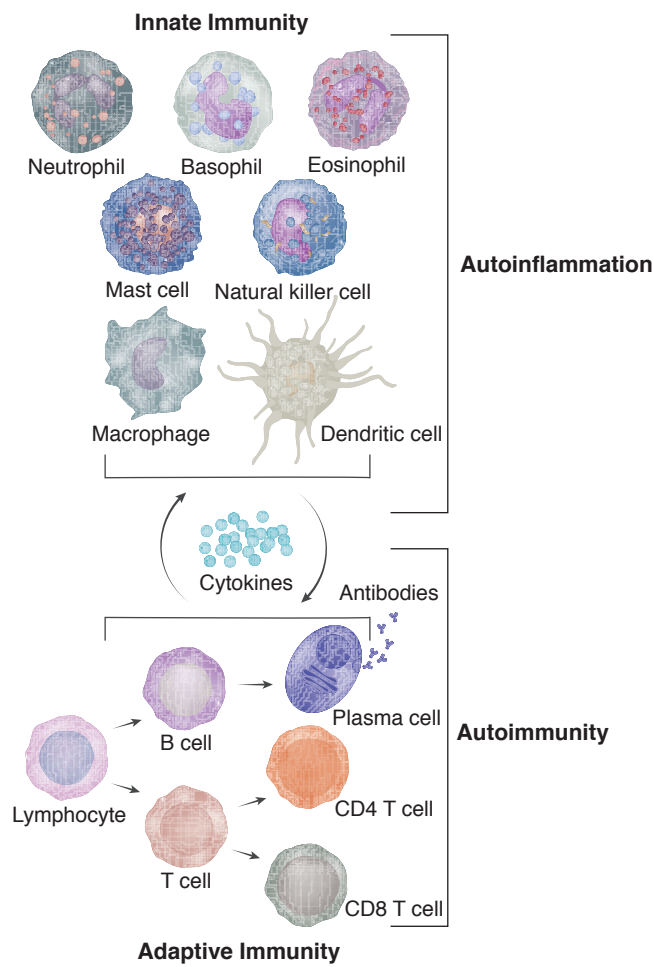


Figure 1-1 Cells of the immune system. (Original illustration by Jared E. Knickelbein, MD, PhD. Redrawn by Cyndie C. H. Wooley.)

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Neutrophils

Neutrophils possess a multilobed nucleus of varying shapes; hence, they are also called *polymorphonuclear leukocytes* or *polymorphonuclear neutrophils* (PMNs). Neutrophils also feature cytoplasmic granules and lysosomes and are the most abundant granulocytes in the blood. They are efficient phagocytes that readily clear tissues, degrade ingested material, and act as important effector cells through the release of granule products and cytokines.

During the beginning or acute phases of inflammation, neutrophils are one of the first inflammatory cells to migrate from the bloodstream toward the site of inflammation. This process is called *chemotaxis*. Neutrophils dominate the inflammatory infiltrate in experimental models and clinical examples of active bacterial infections of the conjunctiva (conjunctivitis), sclera (scleritis), cornea (keratitis), and vitreous (endophthalmitis). They are also dominant in many types of active viral infections of the cornea (eg, herpes simplex virus keratitis) and retina (eg, herpes simplex virus retinitis).

Eosinophils

Eosinophils are characterized by the presence of a bilobed nucleus and abundant lysosomes and cytoplasmic granules that consist of more basic protein than that found in other polymorphonuclear leukocytes. These basic proteins bind acidic dyes, such as eosin—hence the name eosinophil. Eosinophilic granule products, including major basic protein and ribonucleases, destroy parasites efficiently. Eosinophils accumulate at sites of parasitic infection. They are also important in allergic immune reactions. Eosinophilia in the peripheral blood may occur in both parasitic infections and allergic disease.

Eosinophils are abundant in the conjunctiva and tears in many forms of allergic conjunctivitis, especially atopic and vernal conjunctivitis. They are not considered major effectors for intraocular inflammation, with the notable exception of helminthic infections of the eye, especially toxocariasis.

Basophils and Mast Cells

Basophils are the blood-borne equivalent of the tissue-bound mast cell. There are 2 major types of mast cells, connective tissue and mucosal. Both can release preformed granules and synthesize certain mediators *de novo* that differ from those of neutrophils and eosinophils. *Connective tissue mast cells* have abundant granules containing histamine and heparin, and they synthesize prostaglandin D₂ upon stimulation. In contrast, *mucosal mast cells* normally contain low levels of histamine and require T-cell–derived growth-promoting cytokines for stimulation. Stimulated mucosal mast cells primarily synthesize leukotrienes, in particular leukotriene C₄. Tissue location can alter the granule type and functional activity, but regulation of these differences is not well understood.

Mast cells act as major effector cells in immunoglobulin (Ig) E–mediated inflammatory reactions, especially of the allergic or immediate hypersensitivity type. They perform this function through their expression of high-affinity Fc receptors for IgE. *Fc* (from “fragment, crystallizable”) refers to the constant region of immunoglobulin that binds cell surface receptors (see Chapter 2). Mast cells may also participate in the induction of cell-mediated

immunity, wound healing, and other functions not directly related to IgE-mediated degranulation. Other stimuli, such as complement or certain cytokines, may also trigger degranulation.

The healthy human conjunctiva contains numerous mast cells localized in the substantia propria. In certain atopic and allergic disease states, such as vernal conjunctivitis, the number of mast cells increases in the substantia propria and the epithelium, which is usually devoid of mast cells. The uveal tract also contains numerous connective tissue mast cells, whereas the cornea has none.

Monocytes and Macrophages

Monocytes, the circulating cells, and macrophages, the tissue-infiltrating equivalents, are important effectors in innate and adaptive immunity. These mononuclear cells are often detectable in acute ocular infections, even if other cell types, such as neutrophils, are more numerous. Monocytes are relatively large cells (12–20 μm in suspension and up to 40 μm in tissues) that normally travel throughout the body. Most tissues have at least 2 identifiable macrophage populations: tissue resident and blood derived. Although exceptions exist, tissue-resident macrophages are monocytes that migrated into tissue during embryologic development and later acquired tissue-specific properties and cellular markers. Various resident macrophages have tissue-specific names (ie, Kupffer cells in the liver, alveolar macrophages in the lung, and microglia in the brain and retina). Blood-derived macrophages are monocytes that have recently migrated from the blood into a fully developed tissue site.

Macrophages may serve in 3 capacities:

- sentinels that recognize danger signals from pathogens and/or tissue damage
- effectors that induce inflammation and fight pathogens directly
- regulatory/repair cells that conduct tissue repair, regulate the adaptive immune system, and serve as checkpoints during immune cell migration

Various signals can prime resting (immature or quiescent) monocytes for differentiation into efficient antigen-presenting cells (APCs). Upon additional signals, these APCs are activated to become effector cells. Effective activation stimuli include exposure to bacterial products, such as lipopolysaccharide; phagocytosis of antibody-coated or complement-coated pathogens; or exposure to mediators released during inflammation, such as interleukin (IL) 1 β or interferon gamma.

Only after full activation do macrophages become most efficient at the synthesis and release of inflammatory mediators and the killing and degradation of phagocytosed pathogens. Activated macrophages may terminally differentiate into epithelioid cells, with larger nuclei, abundant cytoplasm, and indistinct cell borders, resembling squamous epithelium. These epithelioid histiocytes are characteristic of granulomatous inflammation, either in infectious uveitis (ie, tuberculosis, syphilis, herpes, fungal infection, parasitic uveitis) or noninfectious uveitis (ie, sarcoidosis, granulomatosis with polyangiitis). Activated macrophages may also fuse to form *multinucleated giant cells*, which may accompany granulomatous inflammation or occur in the tissue reaction to foreign material.

Dendritic Cells and Langerhans Cells

Dendritic cells (DCs) are terminally differentiated, bone marrow–derived mononuclear cells that are distinct from macrophages and monocytes. These specialized cells bridge the innate and adaptive immune systems but do not directly participate in effector activities. DCs use pattern recognition receptors, such as Toll-like receptors, to recognize pathogens. Activated DCs upregulate costimulatory molecules and produce cytokines to drive T-cell priming and effector differentiation as well as activate various types of immune cells. Interestingly, antigen presentation by nonactivated, steady-state DCs might lead to T-cell unresponsiveness, promoting tolerance.

All human DCs express high levels of human leukocyte antigen (HLA) class II molecules and may be classified by lineage markers as myeloid/classical or plasmacytoid. DCs can also be classified functionally and anatomically, as their function is linked to their location:

- Blood DCs are precursors of tissue and lymphoid organ DCs.
- Migratory or tissue DCs reside in most epithelial tissues, where they acquire antigen and from which they migrate via afferent lymphatics to lymph nodes. In tissue sites, DCs are large (15–30 μm), with cytoplasmic veils that form extensions 2–3 times the diameter of the cell and resemble the dendritic structure of neurons.
- Resident or lymphoid DCs arise in lymph nodes directly from the blood.
- Inflammatory DCs are present in tissues and lymphoid organs during inflammation. Precursors include classical monocytes.

Langerhans cells (LCs) are myeloid cells with DC function that reside in the epidermis and stratified epithelia of the cornea, as well as conjunctival, buccal, gingival, and genital mucosae. LCs are identified by their many dendrites, electron-dense cytoplasm, and Birbeck granules. Interestingly, they originate from primitive hematopoiesis in the yolk sac and form a stable, self-renewing network that does not require bone marrow–derived precursors in the absence of inflammation. At rest, they are not active APCs. On activation, LCs lose their granules and transform to resemble blood and lymphoid DCs. Evidence suggests that LCs migrate along the afferent lymph vessels to the draining lymphoid organs.

LCs are important components of the immune system and play roles in antigen presentation, control of lymphoid cell traffic, differentiation of T lymphocytes, and induction of delayed hypersensitivity. Elimination of LCs from skin before an antigen challenge inhibits induction of the contact hypersensitivity response. In the conjunctiva and limbus, LCs are the only cells that constitutively express HLA class II molecules. LCs are present in the peripheral cornea, and any stimulation of the central cornea results in central migration of the peripheral LCs.

Lymphocytes

Lymphocytes are small (10–20- μm) cells with large, round, and dense nuclei. Like DCs, they are derived from stem cell precursors within the bone marrow; however, unlike other leukocytes, lymphocytes require subsequent maturation in peripheral lymphoid organs.

Three broad categories of lymphocytes are

- T lymphocytes (also called *T cells*)
- B lymphocytes (also called *B cells*)
- non-T, non-B lymphocytes

The expression of specific cell surface proteins (ie, *surface markers*) can be used to further divide lymphocytes. These markers are in turn related to the functional and molecular activity of individual subsets. Two types of lymphocytes participate in the innate immune response, serving as a bridge between innate and adaptive responses: (1) gamma-delta ($\gamma\delta$) T cells or sentinel T cells, also known as *intraepithelial lymphocytes*; and (2) natural killer (NK) cells, a subset of non-T, non-B lymphocytes. Chapter 2 discusses the roles of lymphocytes in adaptive immunity.

Overview of the Innate Immune System

The innate immune system is a relatively broad-acting rapid reaction force that recognizes nonself (foreign) substances, proteins, or lipopolysaccharides. The innate response is immediate and requires no prior exposure to the foreign substance. Innate immune responses are driven by germline-encoded receptors that recognize features common to many pathogens and offending stimuli. The result is the generation of biochemical mediators and cytokines that recruit innate effector cells, especially macrophages and neutrophils, to remove the offending stimulus through phagocytosis or enzymatic degradation. The innate response also alerts the cells of the adaptive immune system to reinforce and refine the attack. For example, in acute endophthalmitis, bacteria-derived toxins or host cell debris stimulates the recruitment of neutrophils and monocytes, leading to the production of inflammatory mediators and phagocytosis of the bacteria.

An array of highly conserved pattern recognition receptors (PRRs) and proteins detect similarly conserved molecular motifs—*pathogen-associated molecular patterns (PAMPs)*—on triggering stimuli. PAMPs include proteins found in bacterial, fungal, and viral nucleic acids. Cell-associated PRRs may be extracellular, endosomal, or cytoplasmic and include Toll-like receptors (TLRs), C-type lectin receptors (CTLRs), nucleotide-binding oligomerization domain-like receptors (NOD-like receptors, or NLRs), and retinoic acid-inducible gene-I-like receptors (RIG-I-like receptors, or RLRs). Since each PRR has evolved to respond to a different PAMP, engagement of a specific PRR subtype conveys information about the type of infection (ie, bacterial, fungal, or viral) and the location (ie, extracellular or intracellular). In humans, there are 10 members of the TLR family. Those that respond to bacterial products (TLR1/2, TLR2/6, TLR4, TLR5) are localized in the plasma membrane of innate immune cells and sense extracellular microbes. The TLRs that detect viral nucleic acids (TLR3, TLR7, TLR9) are located within endosomal compartments and interact with membrane proteins.

Immunity Versus Inflammation

An immune response is the process for removing an offending stimulus. An immune response that becomes clinically evident is termed an *inflammatory response* (see Clinical

CLINICAL EXAMPLE 1-1**Autoinflammatory and Autoimmune Diseases**

Dysregulation and overactivity of either innate or adaptive immune responses can lead to clinically evident inflammatory disease. Autoimmune disease results from loss of adaptive immune tolerance to auto-antigens (also called *self antigens*) that leads to a dysregulated immune attack on self tissues. Autoreactive B and T cells of the adaptive immune system (discussed further in Chapter 2) are critical to the disease process (see Fig 1-1). An example of a classic autoimmune disease is systemic lupus erythematosus. More recently, the term *autoinflammatory disease* has been used to describe inflammatory conditions driven predominantly by overactivity of the innate immune system in the absence of pathologic B- or T-cell responses. An example of an autoinflammatory disease is juvenile idiopathic arthritis. Most inflammatory diseases likely lie on a spectrum between autoinflammation and autoimmunity. An example of a mixed-spectrum disease is ankylosing spondylitis. The etiology of inflammatory diseases is thought to be multifactorial, with genetic, environmental, and possibly infectious triggers.

Delves PJ, Martin SJ, Burton DR, Roitt IM. *Roitt's Essential Immunology*. 13th ed. Wiley-Blackwell; 2017.

Szekanecz Z, McInnes IB, Schett G, Szamosi S, Benkő S, Szűcs G. Autoinflammation and autoimmunity across rheumatic and musculoskeletal diseases. *Nat Rev Rheumatol*. 2021;17(10):585–595.

Example 1-1). Inflammatory responses typically result in 5 cardinal clinical manifestations: pain, hyperemia, edema, heat, and loss of function. These manifestations are the consequence of 2 physiologic changes within a tissue: cellular recruitment and altered vascular permeability.

The following pathologic findings are typical in inflammation:

- infiltration of effector cells resulting in the release of biochemical and molecular mediators of inflammation, such as cytokines (eg, interleukins and chemokines) and lipid mediators (eg, prostaglandins, leukotrienes, and platelet-activating factors)
- production of oxygen metabolites (eg, superoxide and nitrogen radicals)
- release of granule products as well as catalytic enzymes (eg, proteases, collagenases, and elastases)
- activation of plasma-derived enzyme systems (eg, complement components and fibrin)

These effector processes are described in greater detail later in this chapter.

Immune responses are a constant presence, though usually at a subclinical level. For example, ocular surface allergen exposure, which occurs daily, or the nearly ubiquitous event of bacterial contamination during cataract surgery is usually cleared by immune mechanisms *without* overt inflammation.

Innate Immunity: Triggers and Mechanisms

A variety of triggers and mechanisms are involved in innate immune responses of the eye. Four of the most important triggers are

- bacteria-derived molecules
- damage to nonimmune ocular parenchymal cells by toxins or trauma
- innate mechanisms for the recruitment and activation of neutrophils through the activation of vascular endothelial cells
- innate mechanisms for the recruitment and activation of macrophages

These are discussed in the following sections. Table 1-1 summarizes triggering molecules of innate ocular immune responses.

Bacteria-Derived Molecules That Trigger Innate Immune Responses

Bacterial lipopolysaccharide

Lipopolysaccharide (LPS), also known as *endotoxin*, is an intrinsic component of the cell walls of most gram-negative bacteria. Among the most important triggering molecules of innate immune responses, LPS consists of 3 components: lipid A, O polysaccharide, and core oligosaccharide. Lipid A is the most potent component, capable of activating effector cells at concentrations of a few picograms per milliliter. The exact structures of each component vary among species of bacteria, but all are recognized by the innate immune system. The primary receptors are the TLRs, principally TLR2 and TLR4, which are expressed on macrophages, neutrophils, and DCs, as well as on B cells and T cells.

The effects of LPS include the following:

- activation of monocytes and neutrophils, leading to upregulation of genes for various cytokines (IL-1, IL-6, tumor necrosis factor [TNF])
- degranulation
- activation of complement via the alternative pathway
- activation of vascular endothelial cells

Humans are intermittently exposed to low levels of LPS that the gut releases, especially during episodes of diarrhea and dysentery. Exposure to LPS may play a role in

Table 1-1 Triggering Molecules of Innate Immune Responses in the Eye

Bacteria-derived molecules
Lipopolysaccharide
Other cell wall components
Exotoxins and secreted toxins
Nonspecific soluble molecules (also modulate innate immunity)
Plasma-derived enzymes
Acute-phase reactants
Cytokines produced by parenchymal cells within a tissue site

dysentery-related uveitis, arthritis, and reactive arthritis. LPS is the major cause of shock, fever, and other pathophysiologic responses to bacterial sepsis, making it an important cause of morbidity and mortality during gram-negative bacterial infections. Interestingly, footpad injection of LPS in rodents results in an acute anterior uveitis. This animal model is called *endotoxin-induced uveitis*. See Clinical Example 1-2.

Other bacterial cell wall components

The bacterial cell wall and membrane are complex. They contain numerous polysaccharide, lipid, and protein structures that can initiate an innate immune response independent of adaptive immunity. Killed lysates of many types of gram-positive bacteria or mycobacteria can directly activate macrophages, making them useful as adjuvants. Some of these components have been implicated in various models for arthritis and uveitis. In many cases, the molecular mechanisms might be similar to those of LPS.

CLINICAL EXAMPLE 1-2

Role of Bacterial Toxin Production in the Severity of Endophthalmitis

In experimental models, intraocular injection of lipopolysaccharide (LPS) is highly inflammatory and accounts for much of the enhanced pathogenicity of gram-negative infections of the eye. For example, intravitreal injection of LPS triggers a dose-dependent neutrophilic and monocytic infiltration of the uveal tract, retina, and vitreous. Toll-like receptor 2 (TLR2) recognizes LPS, and binding of LPS by TLR2 on macrophages results in macrophage activation and secretion of a wide array of inflammatory cytokines, including interleukin (IL) 1, IL-6, and tumor necrosis factor α (TNF- α). Degranulation of platelets is among the first histologic changes in LPS-induced uveitis; likely mediators are eicosanoids, platelet-activating factors, and vasoactive amines. The subsequent intraocular generation of several mediators, especially leukotriene B₄, thromboxane B₂, prostaglandin E₂, and IL-6, correlates with the development of the cellular infiltrate and vascular leakage.

Using clinical isolates or bacteria genetically altered to diminish production of the various types of bacterial toxins, investigators have demonstrated that toxin elaboration in gram-positive or gram-negative endophthalmitis greatly influences inflammatory cell infiltration and retinal cytotoxicity. This effect suggests that sterilization through antibiotic therapy alone, in the absence of antitoxin therapy or toxin removal, may not prevent activation of innate immunity, ocular inflammation, and vision loss in eyes infected by toxin-producing strains.

Booth MC, Atkuri RV, Gilmore MS. Toxin production contributes to severity of *Staphylococcus aureus* endophthalmitis. In: Nussenblatt RB, Whitcup SM, Caspi RR, Gery I, eds. *Advances in Ocular Immunology: Proceedings of the 6th International Symposium on the Immunology and Immunopathology of the Eye*. Elsevier; 1994:269–272.

Exotoxins and other secretory products of bacteria

Certain bacteria secrete products known as *exotoxins* into their surrounding microenvironment. Many of these products are enzymes that, although not directly inflammatory, can cause tissue damage and subsequent inflammation and tissue destruction. Examples of these products include

- collagenases
- hemolysins such as streptolysin O, which can kill neutrophils by causing cytoplasmic and extracellular release of their granules
- phospholipases such as the *Clostridium perfringens* α -toxins, which kill cells and cause necrosis by disrupting cell membranes

An intravitreal injection of a purified hemolysin BL toxin derived from *Bacillus cereus* can cause direct necrosis of retinal cells and retinal detachment. In animal studies, as few as 100 *B cereus* organisms can produce enough toxin to cause complete loss of retinal function in 12 hours. In addition to being directly toxic, bacterial exotoxins can be strong triggers of an innate immune response.

Callegan MC, Jett BD, Hancock LE, Gilmore MS. Role of hemolysin BL in the pathogenesis of extraintestinal *Bacillus cereus* infection assessed in an endophthalmitis model. *Infect Immun.* 1999;67(7):3357–3366.

Murphy K, Travers P, Walport M. *Janeway's Immunobiology*. 8th ed. Garland Science; 2012.

Other Triggers or Modulators of Innate Immune Responses

Damage to nonimmune ocular parenchymal cells—especially iris or ciliary body epithelium, retinal pigment epithelium, retinal Müller cells, or corneal or conjunctival epithelium—by toxins or trauma can trigger innate immune responses in the eye. This damage can result in the synthesis of a wide range of mediators, cytokines, and eicosanoids. For example, phagocytosis of staphylococci by corneal epithelium, microtrauma to the ocular surface epithelium by contact lenses, chafing of iris or ciliary epithelium by an intraocular lens, or laser treatment of the retina can stimulate ocular cells to produce mediators that assist in the recruitment of innate effector cells such as neutrophils or macrophages. See Clinical Example 1-3.

CLINICAL EXAMPLE 1-3

Uveitis-Glaucoma-Hyphema Syndrome

One cause of inflammation following cataract surgery, uveitis-glaucoma-hyphema (UGH) syndrome, is related to the physical presence of certain types of intraocular lenses (IOLs). Although UGH syndrome was more common when rigid anterior chamber lenses were used during the early 1980s, it has also been reported with posterior chamber lenses, particularly when a haptic of a 1-piece lens is inadvertently placed in the sulcus. The pathogenesis of UGH syndrome appears related to mechanisms of innate immunity activation. A likely mechanism is cytokine and eicosanoid synthesis triggered by mechanical chafing of or trauma to the iris or ciliary

body. Plasma-derived enzymes, especially complement or fibrin, can enter the eye through vascular permeability altered by surgery or trauma and can then be activated by contact with the surface of IOLs. Adherence of bacteria and leukocytes to the surface has also been implicated. Toxicity caused by contaminants on the lens surface during manufacturing is rare. Nevertheless, noninflamed eyes with IOLs can demonstrate histologic evidence of low-grade, foreign-body reactions around the haptics.

Innate Mechanisms for the Recruitment and Activation of Neutrophils

A key part of the innate immune response is the recruitment and activation of neutrophils, which are highly efficient effectors of this response. Neutrophils are categorized as *resting* or *activated*, according to their secretory and cell membrane activity. In the innate immune response, recruitment of resting, circulating neutrophils occurs rapidly in a tightly controlled process consisting of 2 events:

- neutrophil adhesion to the vascular endothelium through cell adhesion molecules (CAMs) on leukocytes as well as on endothelial cells, primarily in postcapillary venules
- transmigration of the neutrophils through the endothelium and its extracellular matrix, mediated by chemotactic factors

Activation of vascular endothelial cells is triggered by various innate immune stimuli, such as LPS, physical injury, thrombin, histamine, or leukotriene release. *Neutrophil rolling*—a process by which neutrophils bind loosely and reversibly to nonactivated endothelial cells—involves molecules from at least 3 sets of CAM families:

- *selectins*, especially E-, L-, and P-selectin
- *integrins*, especially leukocyte function-associated antigen 1 and macrophage-1 antigen
- *immunoglobulin superfamily molecules*, especially intercellular adhesion molecule (ICAM) 1 and ICAM-2

These molecules are expressed on both neutrophils and vascular endothelial cells.

The primary events are mediated largely by members of the selectin family and occur within minutes of stimulation (Fig 1-2). Nonactivated neutrophils express L-selectin, which mediates a weak bond to endothelial cells by binding to specific selectin ligands. Upon exposure to triggering molecules such as LPS, endothelial cells become activated, expressing in turn at least 2 other selectins (E and P) by which they can bind to the neutrophils and help stabilize the interaction in a process called *adhesion*. Subsequently, other factors, such as platelet-activating factor (PAF), various cytokines, and bacterial products can induce up-regulation of the β -integrin family. As integrins are expressed, the selectins are shed, and neutrophils then bind firmly to endothelial cells through the immunoglobulin superfamily molecules.

After adhesion, various chemotactic factors are required in order to induce *transmigration* of neutrophils across the endothelial barrier and into the extracellular matrix of

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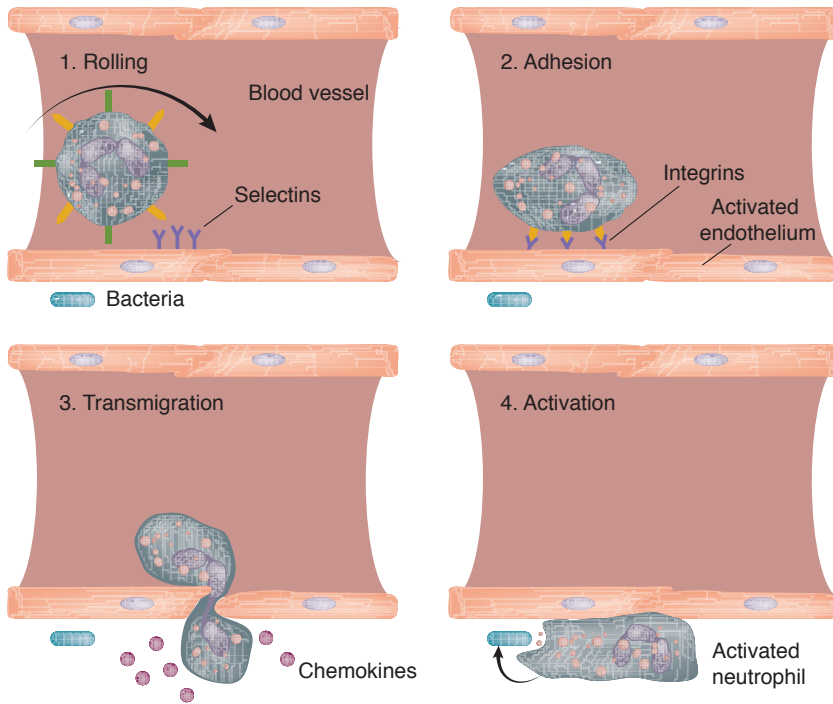


Figure 1-2 Steps of neutrophil migration and activation in infection. **1**, In response to innate immune stimuli, *rolling* neutrophils within the blood vessel bind loosely and reversibly to nonactivated endothelial cells by selectins. **2**, Neutrophil adhesion is mediated by E- and P-selectins, β -integrins, and immunoglobulin superfamily molecules, which are expressed on activated vascular endothelial cells in response to innate activating factors and bacterial products. **3**, Chemotactic factors triggered by the infection induce *transmigration* of neutrophils across the endothelial barrier into the extracellular matrix of the tissue. **4**, Finally, upon stimulation by bacterial toxins and phagocytosis, neutrophils are fully activated, becoming functional effector cells. (Original illustration by Barb Cousins, modified by Joyce Zavarro; redrawn by Cyndie C. H. Wooley.)

the tissue. Chemotactic factors are short-range signaling molecules that diffuse in a declining concentration gradient from the source of production within a tissue to the vessel. Neutrophils have receptors for these molecules and are induced to undergo membrane changes that cause migration in the direction of highest concentration. Examples of chemotactic factors include

- complement products, such as the anaphylatoxin C5a
- fibrin split products
- certain neuropeptides, such as substance P
- bacteria-derived formyl tripeptides, such as *N*-formyl-methionyl-leucyl-phenyl-alanine (fMLP)
- leukotrienes
- α -chemokines, such as IL-8

Activation of neutrophils into functional effector cells begins during adhesion and transmigration but is fully achieved upon neutrophil interaction with specific signals within the site of injury or infection. The most effective activation triggers are bacteria

and their toxins, especially LPS. Other innate or adaptive mechanisms (especially complement) and chemical mediators (such as leukotrienes and PAF) also contribute to neutrophil activation. Neutrophils, unlike monocytes or lymphocytes, do not leave a tissue to recirculate but remain and die.

Phagocytosis

Phagocytosis of bacteria and other pathogens is a process mediated by receptors. The 2 most important are *antibody Fc receptors* and *complement receptors*. Pathogens in an immune complex with antibody or activated complement components bind to cell surface membrane-expressed Fc or complement receptors.

The area of membrane bound by the pathogen invaginates and becomes a phagosome. Cytoplasmic granules and lysosomes then fuse with the phagosomes. Phagocytes have multiple means of destroying microorganisms, notably, antimicrobial polypeptides residing within the cytoplasmic granules as well as reactive oxygen and nitrogen radicals. Although these mechanisms primarily destroy pathogens, released contents, such as lysosomal enzymes, may contribute to the amplification of inflammation and tissue damage.

Neutrophil-derived granule products

As mentioned earlier, neutrophils are proficient phagocytes that act as effector cells through the release of granule products and cytokines. Many antimicrobial polypeptides are present in neutrophilic granules. The principal ones are bactericidal/permeability-increasing protein, defensins, lysozyme, lactoferrin, and the serine proteases.

In addition to antimicrobial polypeptides, neutrophils contain numerous other molecules that contribute to inflammation. These compounds include hydrolytic enzymes, elastase, matrix metalloproteinases (MMPs), gelatinase, myeloperoxidase, vitamin B₁₂-binding protein, and cytochrome *b*₅₅₈. Granule contents remain inert and membrane bound when the granules are intact and become active and soluble when granules fuse to the phagocytic vesicles or plasma membrane. Collagenase is an example of an MMP found within neutrophilic granules. Various forms of collagenase contribute to corneal injury and liquefaction during bacterial keratitis and scleritis, especially in infections with *Pseudomonas* species. Collagenases also contribute to peripheral corneal melting syndromes secondary to rheumatoid arthritis-associated peripheral keratitis.

Innate Mechanisms for the Recruitment and Activation of Macrophages

Monocyte-derived macrophages are another important type of effector cell for the innate immune response that follows trauma or acute infection. The various molecules involved in monocyte adhesion and transmigration from blood into tissues are probably similar to those for neutrophils, although they have not been studied as thoroughly. The functional activation of macrophages, however, is more complex than that of neutrophils. Macrophages exist in different levels or stages of metabolic and functional activity, each representing different “programs” of gene activation and synthesis of macrophage-derived cytokines and mediators. The categories of macrophages include

- resting (immature or quiescent)
- primed

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- activated
- stimulated or reparative (partially activated)

Resting and scavenging macrophages

Phagocytosis removes host cell debris in the process called *scavenging*. Resting macrophages are the classic scavenging cell, capable of phagocytosis and uptake of the following:

- dead cell membranes
- chemically modified extracellular protein (ie, acetylated or oxidized lipoproteins)
- sugar ligands, through mannose receptors
- naked nucleic acids
- bacterial pathogens

Resting monocytes express at least 3 types of scavenging receptors but synthesize very low levels of proinflammatory cytokines. Scavenging can occur in the absence of inflammation. See Clinical Example 1-4.

Primed macrophages

Resting macrophages become primed by exposure to certain cytokines. Upon priming, these cells become positive for HLA class II molecules and capable of functioning as APCs to T lymphocytes (see Chapter 2). Priming involves the following:

- activation of specialized lysosomal enzymes, such as cathepsins D and E, for degrading proteins into peptide fragments
- upregulation of specific genes (ie, major histocompatibility complex class II) and costimulatory molecules (ie, B7.1)
- increased cycling of proteins between endosomes and the cell surface membrane

CLINICAL EXAMPLE 1-4

Phacolytic Uveitis

Mild infiltration of scavenging macrophages centered around retained lens cortex or nucleus fragments occurs in nearly all eyes with lens injury, including those that have undergone routine cataract surgery. This infiltrate is notable for the *absence* of both prominent neutrophil infiltration and substantial nongranulomatous inflammation. An occasional giant cell may be present, but granulomatous changes are not extensive.

Phacolytic uveitis (phacolytic glaucoma) is a variant of scavenging macrophage infiltration in which leakage of lens protein occurs through the intact capsule of a mature or hypermature cataract. Lens protein-engorged scavenging macrophages block the trabecular meshwork outflow channels, resulting in elevated intraocular pressure. Other signs of typical lens-associated uveitis are conspicuously absent. Experimental studies suggest that lens proteins may be chemotactic stimuli for monocytes. See Chapter 8 for further discussion of the clinical presentation of phacolytic uveitis.

Primed macrophages thus resemble DCs. They can exit tissue sites by afferent lymphatic vessels to reenter the lymph node.

Activated and stimulated macrophages

Activated macrophages are classically defined as macrophages that produce the full spectrum of inflammatory and cytotoxic cytokines; thus, they mediate and amplify acute inflammation, tumor killing, and major antibacterial activity. *Epithelioid cells* and *giant cells* represent different terminal differentiations of the activated macrophage.

Activation of macrophages can occur through exposure to innate stimuli or various substances, such as the following:

- cytokines, including chemokines, derived from T lymphocytes and other cell types
- bacterial cell walls or toxins from gram-positive or acid-fast organisms
- complement activated through the alternative pathway
- foreign bodies composed of potentially toxic substances, such as talc or beryllium
- exposure to certain surfaces, such as some plastics

Activation of macrophages is also termed *polarization*. Macrophages can be classified as M1 or M2 based on the observation that certain stimuli produce distinct patterns of gene or protein expression under experimental conditions. For example, exposure to bacterial LPS and interferon gamma typically polarizes macrophages to the M1 phenotype, whereas activation by other innate stimuli in the absence of interferon gamma polarizes toward the M2 phenotype. The activation state seems to be somewhat reversible, suggesting that macrophages may switch between subsets (plasticity), depending on environmental signals. In fact, macrophage research suggests there are at least 9 distinct classes of macrophage activation. Thus, although the M1/M2 model may be oversimplified, it does provide a framework for conceptualizing different levels of macrophage activation in terms of acute inflammation (Fig 1-3).

M1—classically activated macrophage characteristics include

- high production of proinflammatory cytokines (eg, IL-1, IL-6, and TNF- α) and oxygen radicals
- expression of inducible nitric oxide synthase with production of reactive nitrogen intermediates
- promotion of T helper-1 (Th1) response
- strong microbicidal and tumoricidal activity

M2—alternatively activated macrophage characteristics include

- parasite containment
- promotion of tissue remodeling
- promotion of tumor progression
- immunoregulatory functions (eg, arginase and IL-10 secretion)

As noted, macrophages that are partially activated are termed *stimulated* or *reparative* macrophages (M2). Partially activated macrophages contribute to (1) fibrosis and wound healing through the synthesis of mitogens such as platelet-derived growth factors, MMPs,

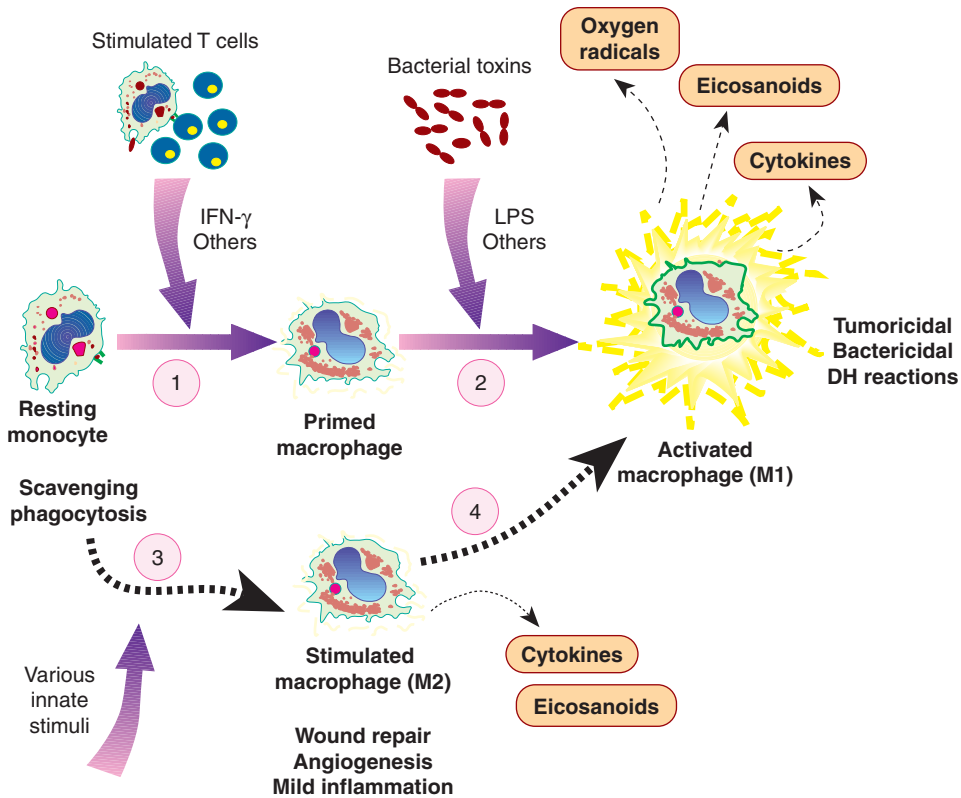


Figure 1-3 Schematic representation of macrophage activation pathway. Classically, *resting monocytes* are thought to be the principal type of noninflammatory scavenging phagocyte. **1**, Upon exposure to low levels of interferon gamma (IFN- γ) from T cells, monocytes become primed, upregulating human leukocyte antigen class II molecules for antigen presentation and performing other functions. **2**, *Fully activated macrophages (M1)*, after exposure to bacterial lipopolysaccharide and interferon (classical activation), are bactericidal and tumoricidal and mediate severe inflammation. **3**, *Stimulated macrophages (M2)* result from resting monocyte activation by other innate stimuli, without exposure to IFN- γ (alternative activation). These cells are incompletely activated, producing low levels of cytokines and eicosanoids but not reactive oxygen intermediates. They participate in wound healing and angiogenesis and have immunoregulatory functions. **4**, Macrophages may switch between subsets (plasticity), depending on environmental signals. DH = delayed hypersensitivity; LPS = lipopolysaccharide. (Illustration by Barb Cousins, modified by Joyce Zavarro.)

and other matrix degradation factors; and to (2) angiogenesis through synthesis of angiogenic factors such as vascular endothelial growth factor.

Phagocyte Killing Mechanisms

Reactive oxygen intermediates

Under certain conditions, oxygen can undergo chemical modification into highly reactive substances with the potential to damage cellular molecules and inhibit functional properties in pathogens or host cells. Three of the most important oxygen intermediates are the

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superoxide anion, hydrogen peroxide, and the hydroxyl radical. Oxygen metabolites triggered by immune responses and generated by leukocytes, especially neutrophils and macrophages, are the most important source of free radicals during inflammation. Leukocyte oxygen metabolism can be initiated by a wide variety of stimuli, including

- innate triggers, such as LPS or fMLP
- adaptive effectors, such as complement-fixing antibodies or certain cytokines produced by activated T cells
- other chemical mediator systems, such as C5a, PAF, and leukotrienes

Reactive oxygen intermediates can also be generated as part of noninflammatory cellular biochemical processes, especially by electron transport in the mitochondria, detoxification of certain chemicals, or interactions with environmental light or radiation. These reactive intermediates are highly toxic to living pathogens and damage pathogenic mediators such as exotoxins and lipids.

Reactive nitrogen products

Nitric oxide (NO) is a highly reactive chemical species. Like reactive oxygen intermediates, NO is involved in various important biochemical functions in microorganisms and host cells. At high concentrations, NO has direct cytotoxic effects on pathogens. The formation of NO depends on the enzyme nitric oxide synthetase (NOS), which is in the cytosol and dependent on NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate). Several types of NOS are known, including various forms of constitutive NOS and inducible NOS (iNOS). Activation induces enhanced production of NO in certain cells, especially macrophages, via the calcium-independent, induced synthesis of iNOS. Many innate and adaptive stimuli modulate induction of iNOS, especially cytokines and bacterial toxins.

Mediator Systems That Amplify Immune Responses

Although innate or adaptive effector responses may directly induce inflammation, in most cases this process must be amplified to produce overt clinical manifestations. Molecules generated within the host that induce and amplify inflammation are termed *inflammatory mediators*, and mediator systems include several categories of these molecules (Table 1-2). Most act on target cells through receptor-mediated processes, although some act in enzymatic cascades that interact in a complex fashion.

Table 1-2 Mediator Systems That Amplify Innate and Adaptive Immune Responses

Plasma-derived enzyme systems: complement, kinins, and fibrin
Vasoactive amines: serotonin and histamine
Lipid mediators: eicosanoids and platelet-activating factors
Cytokines
Neutrophil-derived granule products

Plasma-Derived Enzyme Systems

Complement

Complement is an important inflammatory mediator in the eye. Complement components account for approximately 5% of plasma proteins and comprise more than 30 different proteins. Complement is activated by 1 of 3 pathways, and this activation generates products that contribute to the inflammatory process (Fig 1-4):

- *Classical pathway.* Activation occurs upon fixation of C1 by antigen–antibody (immune) complexes formed by IgM, IgG1, or IgG3. This pathway results in a connection between innate and adaptive immunity.
- *Alternative pathway.* Activation occurs continuously but is restricted by host complement regulatory proteins.
- *Mannose-binding lectin pathway.* This is activated by certain carbohydrate moieties on the cell wall of microorganisms.

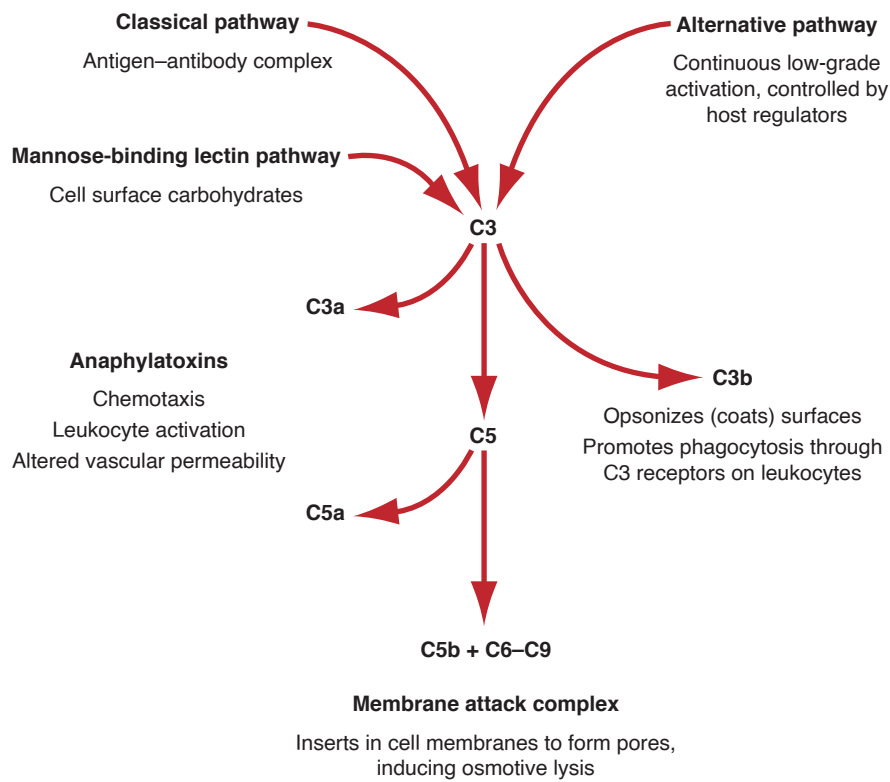


Figure 1-4 Overview of the essential intermediates of the complement pathway. C3a, C3b, C5a, and C5b are complement split products. C5b combines with intact C6, C7, C8, and C9 from the serum.

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Complement serves the following 4 basic functions during inflammation:

- coats antigenic or pathogenic surfaces with C3b to enhance phagocytosis (opsonization)
- promotes lysis of cell membranes through pore formation by the membrane attack complex
- recruits neutrophils and induces inflammation through generation of the anaphylatoxins C3a and C5a
- modulates adaptive immune responses (effects on B and T cells)

Anaphylatoxin effects include chemotaxis, changes in cell adhesiveness, and degranulation and release of mediators from mast cells and platelets. C5a stimulates oxidative metabolism and the production and release of toxic oxygen radicals from leukocytes, as well as the extracellular discharge of leukocyte granule contents.

Defendi F, Thielens NM, Clavarino G, Cesbron JY, Dumestre-Pérard C. The immunopathology of complement proteins and innate immunity in autoimmune disease. *Clin Rev Allergy Immunol.* 2020;58(2):229–251.

Walport MJ. Complement. First of two parts. *N Engl J Med.* 2001;344(14):1058–1066.

Walport MJ. Complement. Second of two parts. *N Engl J Med.* 2001;344(15):1140–1144.

Fibrin and other plasma factors

Fibrin is the final deposition product of the coagulation pathway. Its deposition during inflammation promotes hemostasis, fibrosis, angiogenesis, and leukocyte adhesion. Fibrin is released from its circulating zymogen precursor, *fibrinogen*, upon cleavage by thrombin. In situ polymerization of smaller units gives rise to the characteristic fibrin plugs or clots. Fibrin dissolution is mediated by *plasmin*. Plasminogen is converted to its active form (plasmin) after cleavage by a tissue plasminogen activator. Thrombin, which is derived principally from platelet granules, is released after any vascular injury that causes platelet aggregation and release. Fibrin may be observed in severe anterior uveitis (the “plasmoid aqueous”), and it contributes to complications such as synechiae, cyclitic membranes, and traction retinal detachment.

Histamine

Histamine is present in the granules of mast cells and basophils and is actively secreted after exposure to a wide range of stimuli. Histamine acts by binding to 1 of at least 3 known types of receptors that are differentially present on target cells. The best-studied pathway for degranulation is antigen crosslinking of IgE bound to mast cell Fc IgE receptors, but many other inflammatory stimuli can induce histamine secretion, including complement, direct membrane injury, and certain drugs. Classically, histamine release has been associated with allergy. The contribution of histamine to intraocular inflammation remains subject to debate.

Lipid Mediators

Two groups of lipid molecules synthesized by stimulated cells act as powerful mediators and regulators of inflammatory responses: arachidonic acid (AA) metabolites, or

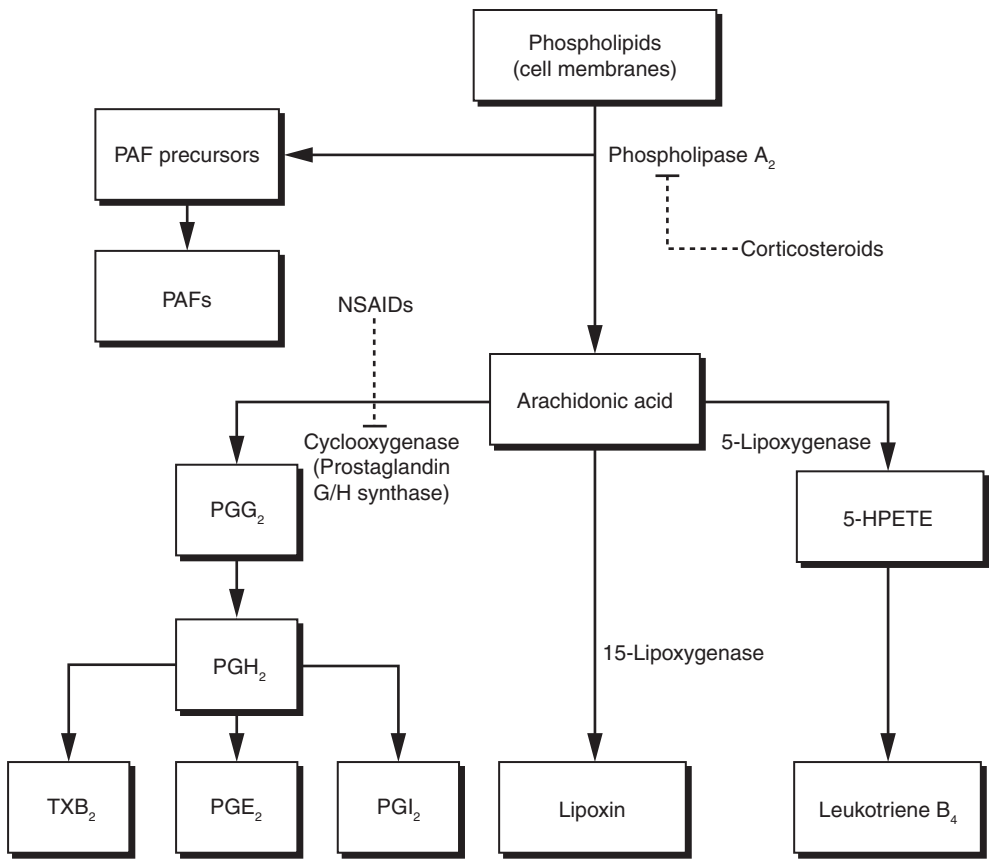


Figure 1-5 Overview of the essential intermediates of the eicosanoid and platelet-activating factor (PAF) pathways. 5-HPETE = 5-hydroperoxyeicosatetraenoic acid; NSAIDs = nonsteroidal anti-inflammatory drugs; PG = prostaglandin; TXB₂ = thromboxane B₂. (Modified with permission from Pepose JS, Holland GN, Wilhelmus KR, eds. *Ocular Infection and Immunity*. Mosby; 1996.)

eicosanoids, and acetylated triglycerides, usually called *platelet-activating factors (PAFs)*. Both groups of molecules may be rapidly generated from the same lysophospholipid precursors by the enzymatic action of cellular phospholipases such as phospholipase A₂ (Fig 1-5).

Eicosanoids

All eicosanoids are derived from AA. AA is liberated from membrane phospholipids by phospholipase A₂, which is activated by various agonists. AA is oxidized by 2 major pathways to generate the various mediators:

- cyclooxygenase (COX) pathway, which produces prostaglandins, thromboxanes, and prostacyclins
- 5-lipoxygenase pathway, which produces 5-hydroperoxyeicosatetraenoic acid, lipoxins, and leukotrienes

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Two forms of COX have been identified. COX-1 is thought to be constitutively expressed by many cells. COX-2 is inducible by various inflammatory stimuli (eg, LPS, PAF, and some cytokines). The COX-derived products are evanescent compounds induced in virtually all cells by a variety of stimuli. In general, they act in the immediate environment of their release to directly mediate many inflammatory activities. These include effects on vascular permeability, cell recruitment, platelet function, and smooth-muscle contraction. Prostaglandins may play a role in uveitic macular edema in association with anterior segment surgery or inflammation. Posterior diffusion of 1 or more of the eicosanoids through the vitreous is assumed to alter the permeability of the perifoveal capillary network, leading to macular edema. Clinical trials in humans suggest that topical treatment with COX inhibitors (eg, nonsteroidal anti-inflammatory drugs [NSAIDs]) reduces the incidence of macular edema after cataract surgery.

Derivatives of 5-lipoxygenase, an enzyme found mainly in granulocytes and some mast cells, have been detected in the brain and retina. Some leukotrienes have 1000 times the effect of histamine on vascular permeability. Another lipoxygenase product, lipoxin, is a potent stimulator of superoxide anion. Because many of the COX-derived prostaglandins downregulate the lipoxygenase pathway, NSAIDs can tilt AA metabolism toward increased production of inflammatory metabolites, leukotrienes, and lipoxins.

Platelet-activating factors

Platelet-activating factors are a family of phospholipid-derived mediators that appear to be important stimuli in the early stage of inflammation. Phospholipase A₂ metabolizes phosphocholine precursors in cell membranes, releasing AA and PAF precursors, which are acetylated into multiple species of PAF. PAF release is stimulated by various innate immune triggers, such as bacterial toxins, trauma, and cytokines. PAFs activate not only platelets but also most leukocytes, which in turn produce and release additional PAFs. PAFs function by binding to 1 or more guanosine triphosphate protein-associated receptors on target cells.

In vitro, PAFs induce an impressive repertoire of responses, including phagocytosis, exocytosis, superoxide production, chemotaxis, aggregation, proliferation, adhesion, eicosanoid generation, degranulation, and calcium mobilization, as well as diverse morphologic changes. PAFs are a major regulator of cell adhesion and vascular permeability in many forms of acute inflammation, trauma, shock, and ischemia. The precise role of PAFs in intraocular inflammation remains unknown, but synergistic interactions probably exist among PAFs, nitric oxide, eicosanoids, and cytokines. However, intravitreal injection of PAFs in animals induces an acute retinitis and photoreceptor toxicity.

Cytokines

Cytokines are soluble polypeptide mediators synthesized and released by cells for the purposes of intercellular signaling and communication, both within the innate immune system and between the innate and adaptive immune systems. Various types of intercellular signaling occur, including *paracrine* (signaling of neighboring cells at the same site), *autocrine* (stimulation of a receptor on its own surface, and *endocrine* (action on a distant site through release into the blood). Table 1-3 lists examples of cytokines associated with ocular inflammation.

Table 1-3 Cytokines of Relevance to Ocular Immunology

Family	Example	Major Cell Source	Major Target Cells	Major General Actions	Specific Ocular Actions/Clinical Relevance
Interleukins (ILs)	IL-1 β	Monocytes	Most leukocytes Various ocular cells	Induces cyclooxygenase type 2 leading to fever, vasodilatation, hypotension (shock)	Altered vascular permeability
		Macrophages		Promotes infiltration of inflammatory cells into extravascular space and tissues	Neutrophil and macrophage infiltration
		Neutrophils		Promotes angiogenesis	Langerhans cell migration to central cornea
		Dendritic cells		Induces IL-6 and IL-17 expression	Elevated levels in aqueous and serum in many forms of uveitis
		T cells		Activates CD4 and CD8 T lymphocytes	Detectable levels in some forms of uveitis
	IL-2	Th0 or Th1 CD4 T lymphocytes	T lymphocytes B lymphocytes NK cells	Induces Th1	Role in atopic and vernal conjunctivitis
		Th2 CD4		Induces Th2, blocks Th1	
		T lymphocytes		Induces B lymphocytes to synthesize immunoglobulin E	
	IL-4	Basophils, mast cells	Eosinophils	Recruits eosinophils	Role in atopic and vernal conjunctivitis
		Th2 CD4		Has many actions on B lymphocytes, including enhancement of antibody production	
		T lymphocytes		Induces T-cell polarization	
	IL-5	Mast cells	Most leukocytes Various ocular cells	Induces systemic toxicity (fever, shock, production of acute-phase proteins in liver)	Altered vascular permeability
		Endothelium			
	IL-6	Monocytes	Various ocular cells		Neutrophil infiltration
		Macrophages			
		T lymphocytes			
	IL-6	Mast cells			High levels in serum, aqueous, and vitreous in many forms of uveitis and nonuveitic diseases
		Endothelium			

Family	Example	Major Cell Source	Major Target Cells	Major General Actions	Specific Ocular Actions/Clinical Relevance
α -Chemokines	IL-12/23 (IL-12 family)	Macrophages Dendritic cells B lymphocytes (IL-23) Endothelium (IL-23)	Naive CD4 T lymphocytes	Key Th1-inducing cytokine Activates NK cells Mediates chronic inflammation through promotion of Th17 lymphocytes	High expression in aqueous and vitreous from idiopathic uveitis cases High expression in serum in Behçet disease and VKH syndrome
	IL-17A	T lymphocytes (Th17, gamma-delta [$\gamma\delta$]) NK T lymphocytes	Most leukocytes Various ocular cells	Key cytokine of Th17 response, driving inflammation/tissue damage Induces proinflammatory cytokines, chemokines, and adhesion molecules Important driver of autoimmunity	High expression in patients with active uveitis, noninfectious (Behçet disease, birdshot chorioretinopathy, HLA-B27 uveitis, VKH syndrome) and infectious (toxoplasmic retinochoroiditis, viral retinitis) Leads to disruption of outer blood–retina barrier Role in infectious keratitis High expression in inflamed eye Altered vascular permeability Neutrophil infiltration
	IL-8/CXCL8	Many cell types	Endothelial cells Neutrophils Many others	Recruits and activates neutrophils Upregulates CAM on endothelium Chemotactic for basophils and T lymphocytes Promotes angiogenesis	
β -Chemokines	Macrophage chemotactic protein-1/CCL2	Macrophages Endothelium RPE	Endothelial cells Macrophages T lymphocytes	Recruits and activates macrophages, some T lymphocytes	High expression in noninflamed and inflamed eyes Recruits macrophages and T lymphocytes to eye
Tumor necrosis factors (TNFs)	TNF- α or TNF- β	Macrophages T lymphocytes	Most leukocytes Various ocular cells	Tumor apoptosis Macrophage and neutrophil activation Cell adhesion and chemotaxis Fibrin deposition and vascular injury Systemic toxicity (fever, shock)	Altered vascular permeability Mononuclear cell infiltration

(Continued)

Table 1-3 (continued)

Family	Example	Major Cell Source	Major Target Cells	Major General Actions	Specific Ocular Actions/Clinical Relevance
Interferons (IFNs)	IFN- γ	Th1 cells NK cells	Macrophages Dendritic cells	Activates macrophages Facilitates Th1 development Mediates delayed-type hypersensitivity reactions	Neutrophil and macrophage infiltration MHC II upregulation on iris and ciliary epithelium, RPE
	IFN- α	Most leukocytes	Most parenchymal cells	Prevents viral infection of many cells Inhibits hemangioma, conjunctival intraepithelial neoplasia, and other tumors	Innate protection of ocular surface from viral infection Treatment of ocular surface neoplasms
Growth factors	Transforming growth factor β family (TGF- β)	Leukocytes RPE and NPE of ciliary body Pericytes Fibroblasts	Macrophages T lymphocytes RPE Glial cells	Regulates immune response, suppresses T-lymphocyte and macrophage inflammatory functions	High expression in the noninflamed eye Regulator of immune privilege and ACAID
		Platelets Macrophages RPE	Fibroblasts Fibroblasts Glial cells Many others	Regulates wound repair: fibrosis Fibroblast proliferation	Role in inflammatory membranes, subretinal fibrosis
	Vascular endothelial growth factor (VEGF) family	Macrophages Platelets Several retinal cells (RPE, astrocytes, Müller cells, vascular endothelium)	Vascular endothelial cells Leukocytes	Neovascularization and vascular permeability Leukocyte recruitment	Role in retinovascular disease (diabetic macular edema, exudative AMD, PDR, retinal vein occlusion, ROP) Corneal neovascularization and nerve growth
		Ocular nerves	Leukocytes Others	Pain	Altered vascular permeability Leukocyte infiltration
Neuropeptides	Substance P	Ocular nerves	Leukocytes Others	Altered vascular permeability Suppresses macrophage and T-lymphocyte inflammatory function	Role in ACAID and immune privilege
	Vasoactive intestinal peptide	Ocular nerves	Leukocytes Others		

ACAID = anterior chamber-associated immune deviation; AMD = age-related macular degeneration; CAM = cell adhesion molecules; CCL = chemokine ligand; CXCL = C-X-C motif chemokine ligand; HLA = human leukocyte antigen; MHC = major histocompatibility complex; NK = natural killer; NPE = nonpigmented epithelium; PDR = proliferative diabetic retinopathy; RPE = retinal pigment epithelium; ROP = retinopathy of prematurity; Th = T helper; VKH = Vogt-Koyanagi-Harada.

Traditionally, investigators have divided cytokines into families with related activities, sources, and targets, using terms such as *growth factors*, *interleukins*, *lymphokines*, *interferons*, *monokines*, and *chemokines*. Thus, *growth factor* traditionally refers to cytokines that mediate cell proliferation and differentiation. The terms *interleukin* and *lymphokine* identify cytokines thought to mediate intercellular communication among lymphocytes or other leukocytes. Interferons are cytokines that limit or interfere with the ability of a virus to infect a cell. Monokines are immunoregulatory cytokines secreted by monocytes and macrophages. Chemokines are chemotactic cytokines. Although some cytokines are specific for particular cell types, most have high degrees of multiplicity and redundancy of source. For example, activated macrophages in an inflammatory site synthesize growth factors, interleukins, interferons, and chemokines.

Both innate and adaptive responses result in the production of cytokines. T lymphocytes are the classic cytokine-producing cell of adaptive immunity, but macrophages, mast cells, and neutrophils also synthesize a wide range of cytokines upon stimulation. Cytokine interactions can be additive, combinatorial, synergistic, or antagonistic. Elimination of the action of a single molecule may have an unpredictable outcome. For example, monoclonal antibodies directed against TNF- α result in substantial suppression of immune responses but also increase susceptibility to multiple sclerosis. Finally, cytokines not only act as mediators and amplifiers of inflammation in innate and adaptive immune responses but also modulate the initiation of immune responses; the function of most leukocytes is altered by preexposure to various cytokines. Thus, for many cytokines, their regulatory role may be as important as their actions as mediators of inflammation.

Targeting cytokines with monoclonal antibodies or soluble receptors has become an important and highly effective therapeutic strategy for combating inflammatory diseases (see Table 1-4 on the following pages). For example, TNF- α is a potent proinflammatory cytokine produced predominantly by activated monocytes, macrophages, and T cells that plays a role in the pathogenesis of several autoimmune diseases. Multiple TNF- α blockers are currently approved by the US Food and Drug Administration for the treatment of a number of inflammatory diseases, including noninfectious intermediate uveitis, posterior uveitis, and panuveitis (also see Chapter 6).

Neutrophils and Their Products

Neutrophils are a source of specialized products that can amplify immune responses. These products are discussed in the section “Neutrophil-derived granule products.”

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Table 1-4 Clinically Relevant Cytokine Inhibitors					
Cytokine Targeted	Drug Examples	Drug Description	Route of Administration	FDA-Approved Indication	Ocular Relevance/Use
IL-1 β	Anakinra	IL-1 receptor antagonist	Subcutaneous injection	Rheumatoid arthritis and several rare autoinflammatory conditions	Off-label use in Behçet disease
IL-6	Canakinumab	Human monoclonal Ab	Subcutaneous injection	JIA and periodic fever syndromes	Off-label use in Behçet disease
	Tocilizumab	Humanized monoclonal Ab	IV infusion or subcutaneous injection	Rheumatoid arthritis, giant cell arteritis, JIA, cytokine release syndrome	Off-label use in noninfectious intermediate, posterior, and panuveitis
IL-17A	Secukinumab	Human monoclonal Ab	Subcutaneous injection	Plaque psoriasis and psoriatic arthritis, ankylosing spondylitis	Failed to show efficacy in treatment of noninfectious intermediate, posterior, and panuveitis in randomized clinical trial
TNF- α	Adalimumab	Human monoclonal Ab	Subcutaneous injection	Rheumatoid arthritis; JIA; plaque psoriasis and psoriatic arthritis; ankylosing spondylitis; Crohn disease; ulcerative colitis; hidradenitis suppurativa; noninfectious intermediate, posterior, and panuveitis	On-label use in noninfectious intermediate, posterior, and panuveitis
	Infliximab	Chimeric monoclonal Ab	IV infusion	Crohn disease, ulcerative colitis, rheumatoid arthritis, ankylosing spondylitis, plaque psoriasis, psoriatic arthritis	Off-label use in noninfectious intermediate, posterior, and panuveitis

Cytokine Targeted	Drug Examples	Drug Description	Route of Administration	FDA-Approved Indication	Ocular Relevance/Use
VEGF-A	Bevacizumab	Humanized monoclonal Ab	IV infusion for FDA-approved indications; intravitreal injection for off-label ocular use	Metastatic colorectal cancer and several other cancers	Off-label use in retinovascular disease (neovascular AMD, diabetic retinopathy, diabetic macular edema, ROP, RVO) and refractory uveitic macular edema
	Ranibizumab	Humanized monoclonal Ab	Intravitreal injection	Neovascular AMD, macular edema following RVO, diabetic macular edema, diabetic retinopathy, myopic CNV	In addition to FDA indications, off-label use in refractory uveitic macular edema
	Aflibercept	Fusion protein with human VEGF receptors 1 and 2 fused to human Fc	Intravitreal injection	Neovascular AMD, macular edema following RVO, diabetic macular edema, diabetic retinopathy	In addition to FDA indications, off-label use in refractory uveitic macular edema

Ab = antibody; AMD = age-related macular degeneration; CNV = choroidal neovascularization; Fc = fragment, crystallizable; FDA = Food and Drug Administration; IL = interleukin; IV = intravenous; JIA = juvenile idiopathic arthritis; ROP = retinopathy of prematurity; RVO = retinal vein occlusion; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

Immunization and Adaptive Immunity: The Immune Response Arc and Immune Effectors

Highlights

- Adaptive (or acquired) immunity involves a “learned” response to specific antigens rather than a response to conserved molecular patterns.
- The adaptive immune system continuously samples antigenic epitopes, determines whether they are self or nonself, and then mounts an immune response to eliminate foreign antigens.
- Conversion of an antigenic stimulus to an immune response involves activation of immune cells, particularly T and B lymphocytes, and development of immunologic memory for that specific antigen.
- The adaptive immune response comprises antibody-mediated responses, cell-mediated defense primarily coordinated by T lymphocytes, and combined antibody and cellular mechanisms.
- Cell-mediated responses (particularly T helper [Th]-1, Th2, and Th17 responses) are relevant to the immunopathology of several intraocular inflammatory conditions.

Definitions

The term *antigen* refers to a substance recognized by the immune system. The term *epitope* refers to each specific portion of an antigen to which an antibody molecule can bind. Antigenic epitopes exist on both native self tissues and foreign, or nonself, tissues. A complex, 3-dimensional protein has multiple antigenic epitopes that the immune system can recognize, as well as many other sites that remain unrecognizable and thus invisible to the immune system.

The adaptive immune response, unlike the innate immune response (discussed in Chapter 1), is a learned response to highly variable but specific antigenic epitopes rather

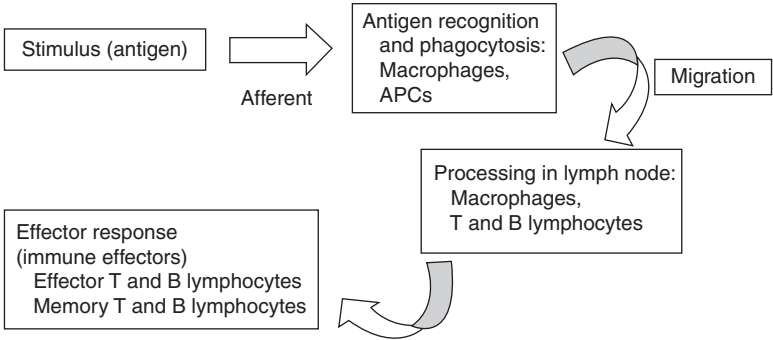


Figure 2-1 The immune response arc. APCs=antigen-presenting cells.

than a response to conserved molecular patterns. The purpose of the adaptive immune system is to continuously sample antigenic epitopes, determine whether they are self or non-self, and then mount an immune response to eliminate foreign antigens. The immune response arc—the interaction between antigen and the adaptive immune system—can be divided into 3 phases: afferent, processing, and effector (Fig 2-1).

Phases of the Immune Response Arc

Afferent Phase

The afferent phase of the immune response arc comprises the initial recognition, transport, and presentation of antigenic substances to the adaptive immune system. Recognition starts with antigen-presenting cells (APCs), specialized cells that bind antigen at a peripheral site. After the APC receives stimulatory signals (ie, complement), phagocytosis of the antigen occurs, as discussed in Chapter 1. Following ingestion of antigen, APCs migrate via afferent lymphatics to regional lymph nodes.

In the APCs, enzymatic digestion of proteins within endocytic vesicles produces antigenic epitope fragments of 7–11 amino acids (Fig 2-2). Each antigenic fragment binds to a groove-shaped human leukocyte antigen (HLA) peptide residing on the APC surface. The combination of antigenic peptide and HLA protein is recognized by the T-lymphocyte receptors CD4 and CD8. HLA molecules differ in their capacity to bind various antigenic peptide fragments within their grooves; thus, the HLA type determines the array of peptide antigens that can be presented to T lymphocytes. Specific HLA alleles are important risk factors for certain forms of uveitis. See Chapter 4 for further discussion of HLA molecules and disease susceptibility.

HLA peptides are part of a family of cell surface glycoproteins called *major histocompatibility complex (MHC) proteins*. In humans, MHC proteins are termed *human leukocyte antigen (HLA) molecules*. HLA class I molecules (ie, HLA-A, -B, and -C) serve as the antigen-presenting platform for CD8⁺ T lymphocytes, which are essentially cytotoxic T cells (see Fig 2-2A). Class I molecules are present on almost all nucleated cells and generally function to process peptide antigens synthesized by the host cell. If these presented antigens are

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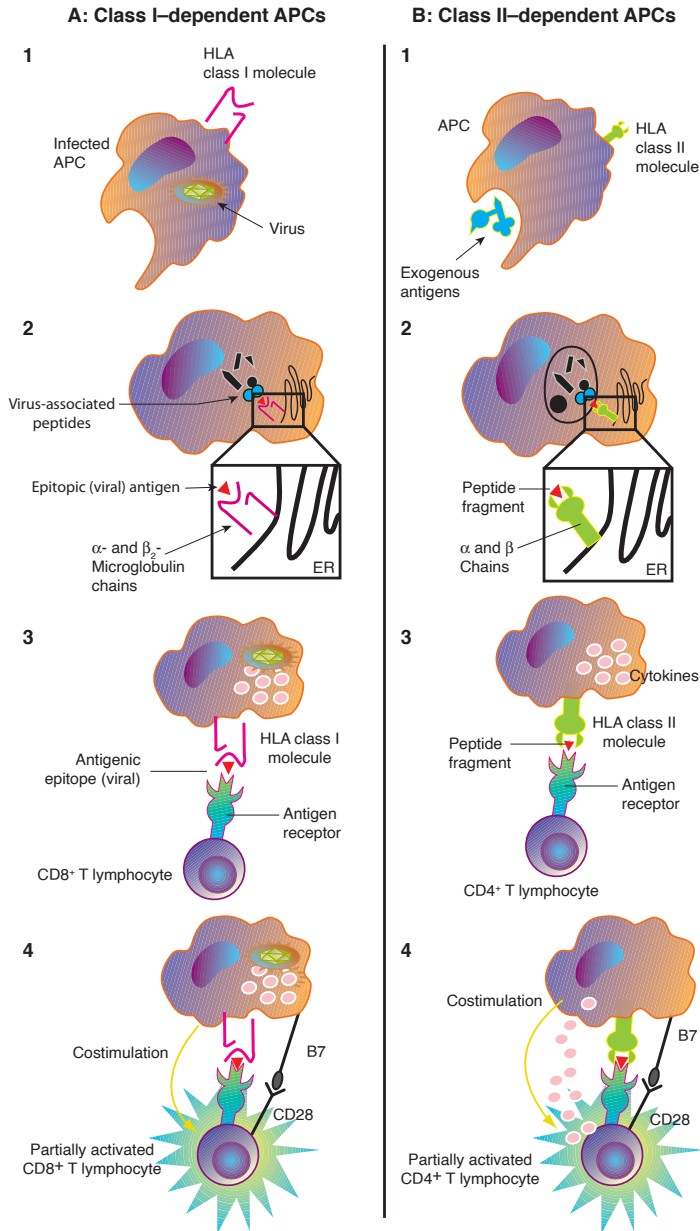


Figure 2-2 A, Class I-dependent APCs. **1,** APC is infected by a virus, which causes the cell to synthesize virus-associated peptides that are present in the cytosol. **2,** The viral antigen is transported through specialized transport systems into the endoplasmic reticulum (ER), where the viral antigen encounters human leukocyte antigen (HLA) class I molecules. The antigenic fragment binds to the groove formed by the α chain of the HLA class I molecule. Unlike class II molecules, the second chain, called β_2 -microglobulin, is constant among all class I molecules. **3,** The CD8 T-lymphocyte receptor recognizes the fragment–class I complex displayed on the cell membrane. **4,** With the help of costimulatory molecules such as CD28–B7 and cytokines, the CD8⁺ T lymphocyte becomes primed, or partially activated. A similar mechanism is used to recognize tumor antigens that are produced by cells after malignant transformation. **B, Class II-dependent APCs.** **1,** APCs endocytose exogenous antigens into the ER. **2,** In the ER, the antigen is digested, generating peptide fragments that bind to the groove formed by the α and β chains of the HLA class II molecule. **3,** The CD4⁺ T-lymphocyte receptor recognizes the fragment–class II complex. **4,** With the help of costimulatory molecules such as CD28–B7 and cytokines, the CD4⁺ T lymphocyte becomes primed, or partially activated. (Illustration by Barb Cousins, modified by Joyce Zavarro.)

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recognized as self (ie, normal host protein), no immune reaction occurs. However, if there is an alteration of the normal host peptide (termed *altered self*), by tumor or viral peptides after host cell invasion, an immune response is initiated. A viral infection, a neoplasm, or simply a genetic mutation that alters protein structure may induce autoimmunity by stimulating an inappropriate immune response to normal host proteins.

HLA class II molecules (ie, HLA-DR, -DP, and -DQ) serve as the antigen-presenting platform for CD4⁺, or *helper*, T lymphocytes (see Fig 2-2B). The antigen receptor on the helper T lymphocyte recognizes peptide antigens only if the antigens are presented with class II molecules simultaneously. Only certain cell types express HLA class II molecules. Macrophages and dendritic cells are the most important of these types, although B lymphocytes also may function as class II-dependent APCs, especially within a lymph node. Class II-dependent APCs are considered the most efficient APCs for processing extracellular protein antigens; that is, antigens that have been phagocytosed from the external environment (eg, bacterial or fungal antigens).

Processing Phase

The conversion of an antigenic stimulus into an immunologic response occurs through *priming* of naive B and T lymphocytes (lymphocytes that have not yet encountered their specific antigen) within lymph nodes and the spleen. Processing involves regulation of the interaction between antigen and naive lymphocytes, followed by lymphocyte *activation*, which consists of lymphocyte proliferation and differentiation (Fig 2-3).

Preconditions necessary for processing

The principal cell type for immune processing is the CD4⁺ T lymphocyte. These lymphocytes have a receptor that detects antigen only upon formation of a trimolecular complex consisting of an HLA class II molecule, a processed antigenic fragment, and a T-lymphocyte antigen receptor (see Fig 2-2B). The CD4⁺ molecule stabilizes binding and enhances signaling between the HLA complex on the APC and the T-lymphocyte receptor. When helper T lymphocytes recognize their specific antigen, they become primed, or partially activated, acquiring new functional properties, including cell division, cytokine synthesis, and cell membrane expression of *accessory molecules*, such as cell adhesion molecules and costimulatory molecules. The synthesis and release of immune cytokines, especially interleukin (IL)-2, by T lymphocytes is crucial for the progression of initial activation and the functional differentiation of T lymphocytes through autocrine stimulation.

Helper T-lymphocyte differentiation

Prior to priming, CD4⁺ T lymphocytes are classified as T helper (Th)-0 cells. These cells can differentiate into 1 of at least 4 functional subtypes—Th1, Th2, Th17, or T regulatory (Treg)—based on the pattern of cytokines to which they are exposed (Fig 2-4). Each helper T-cell subtype, in turn, produces a characteristic profile of cytokines that is regulated by the expression of subtype-specific transcription factors.

Th1 cells participate in the elimination of intracellular pathogens as well as in cell-mediated and delayed hypersensitivity reactions. Th1 cells secrete interferon gamma (IFN- γ), IL-2, and tumor necrosis factor α and β (TNF- α , TNF- β).

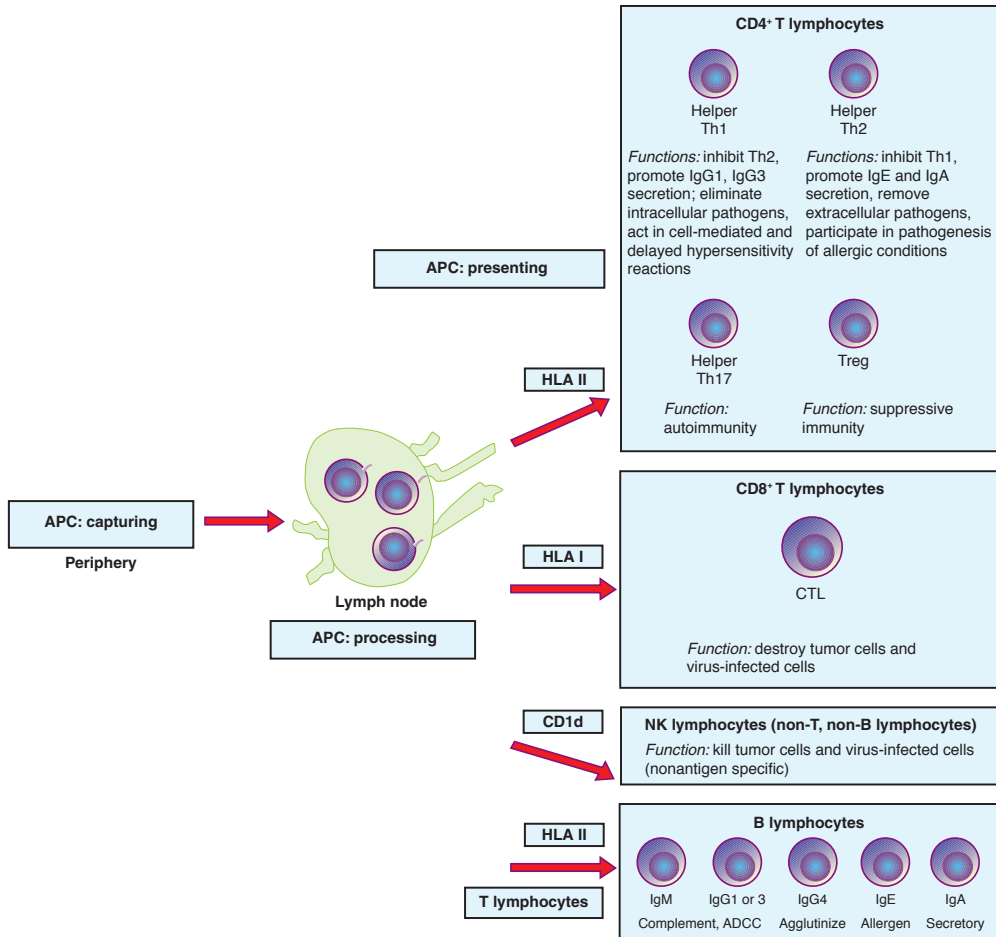


Figure 2-3 Schematic illustration of immune processing of antigen within the lymph node. On exposure to antigen and APCs within the lymph node, the 3 major lymphocyte subsets—B lymphocytes, CD4⁺ T lymphocytes, and CD8⁺ T lymphocytes—and natural killer (NK) (non-T, non-B) lymphocytes are activated to release specific cytokines and perform particular activities. Antigen presented by HLA class II molecules stimulates CD4⁺ T lymphocytes to differentiate into 1 of at least 4 subtypes: T helper (Th)-1, Th2, Th17, or T regulatory (Treg).

Naive CD8⁺ T cells become cytotoxic T lymphocytes (CTLs), and non-T, non-B lymphocytes become NK lymphocytes. B lymphocytes are stimulated to produce 1 of the various antibody isotypes, whose functions may include complement activation, antibody-dependent cellular cytotoxicity (ADCC), agglutination, allergen recognition, and/or secretory release. Ig = immunoglobulin. (Illustration by Barb Cousins, modified by Joyce Zavarro.)

Th2 cells are involved in the clearance of extracellular pathogens and play an important role in the pathogenesis of allergic conditions such as asthma. Th2 cells produce IL-4, IL-5, and IL-13 but not Th1 cytokines.

Th17 cells contribute to immunity against certain extracellular bacteria and fungi and play a role in the defense of mucosal surfaces. Th17 cells produce IL-17, IL-21,

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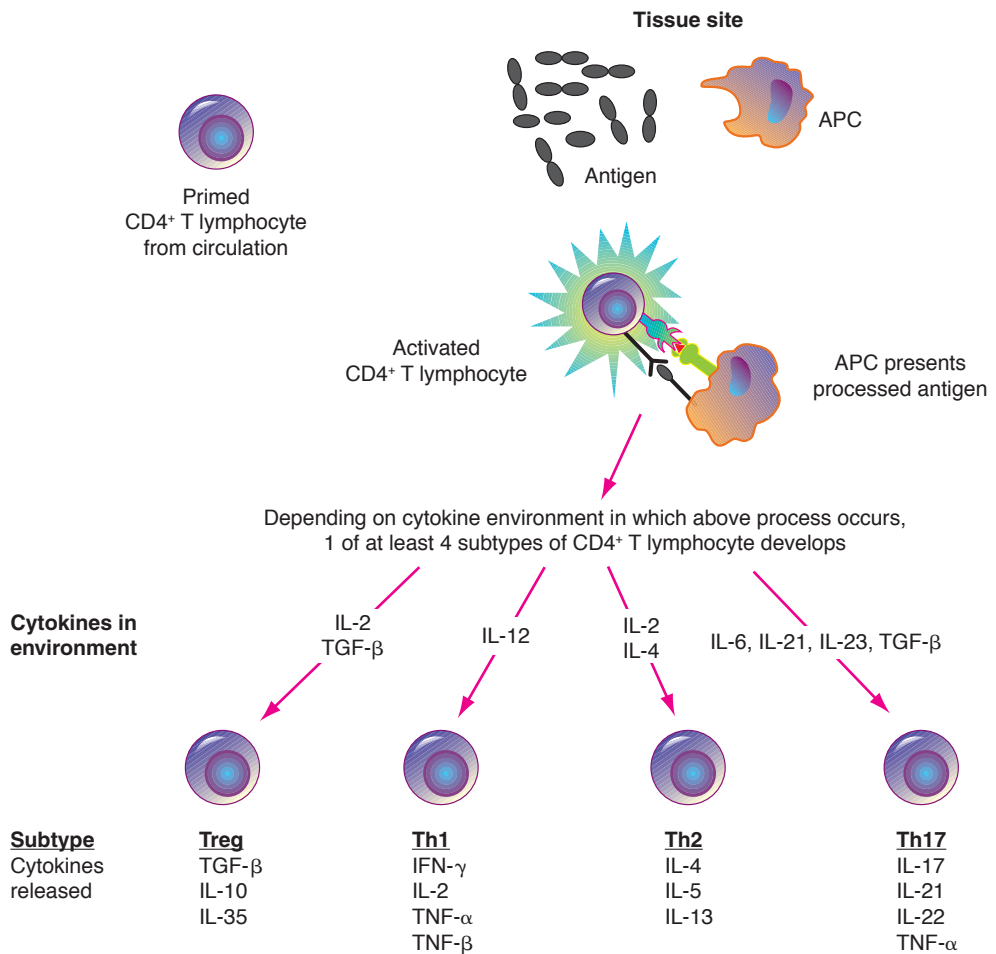


Figure 2-4 Schematic representation of CD4⁺T-lymphocyte development. After initial priming in the lymph node, CD4⁺ T lymphocytes enter the tissue site, where they again encounter APCs containing processed antigen. Upon restimulation and depending on the cytokines present in the local environment at the time of restimulation, CD4⁺ T lymphocytes become activated into 1 of at least 4 subtypes. Tregs suppress other T-cell responses. Th1 lymphocytes are the classic delayed hypersensitivity effector cells and mediate interferon gamma (IFN- γ)–driven responses. Th2 lymphocytes are thought to be less intensively inflammatory and have been associated with granuloma formation in response to parasite-derived antigens, as well as manifestations of atopic diseases. Th17 lymphocytes mediate and sustain inflammation. IL = interleukin; TGF = transforming growth factor. (Illustration developed by Russell W. Read, MD, PhD.)

IL-22, and TNF- α , in association with several transcription factors, including retinoic acid receptor–related orphan receptor- γ t and receptor- α (ROR- γ t and - α). Dysregulation of Th17 proinflammatory cytokines IL-17 and IL-22 has been implicated in the pathogenesis of systemic inflammatory diseases, such as psoriasis, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, Sjögren syndrome, Behçet disease, and systemic lupus erythematosus, as well as in uveitis and scleritis.

Treg cells are essential for maintaining peripheral tolerance to autoantigens (also called *self antigens*), thereby preventing autoimmune diseases and limiting chronic inflammatory diseases. Treg cells suppress excessive immune responses deleterious to the host and downregulate autoreactive T cells. Treg cells are identified by their simultaneous expression of CD4, CD25, and Foxp3. The suppressive cytokines transforming growth factor β and IL-10 have been implicated as active players in the effector function of Treg cells as regulators of inflammation.

An imbalance between regulatory mechanisms that inhibit the immune system and proinflammatory responses is thought to be the underlying cause of uveitis and many other immune-mediated diseases. The cytokine profiles produced by these various helper T-cell subtypes determine subsequent immune processing, B-lymphocyte antibody synthesis, and cell-mediated effector responses. For example, IFN- γ produced by Th1 lymphocytes inhibits the Th2 response, whereas IL-4 produced by Th2 lymphocytes inhibits the Th1 response. The process determining whether a Th1 or a Th2 response develops with exposure to a specific antigen is not entirely understood, but presumed variables include cytokines preexisting in the microenvironment, the nature and amount of antigen encountered, and the type of APC involved. For example, IL-12, which is produced by macrophage APCs, might preferentially induce Th1 responses.

Abbas AK, Lichtman AH, Pillai S. *Cellular and Molecular Immunology*. 9th ed. Elsevier/Saunders; 2018.

Amadi-Obi A, Yu CR, Liu X, et al. Th17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1. *Nat Med*. 2007;13(6):711–718.

Caspi RR. Understanding autoimmunity in the eye: from animal models to novel therapies. *Discov Med*. 2014;17(93):155–162.

B-lymphocyte activation

A major function of helper T lymphocytes is B-lymphocyte activation. B lymphocytes are responsible for producing antibodies, or immunoglobulin (Ig) molecules (glycoproteins that bind to the specific antigens or epitopes that induced their synthesis). These antibodies contain an epitope-specific binding site, termed *paratope*, on the Fab (fragment, antigen-binding) portion of the molecule. B lymphocytes begin as naive lymphocytes; IgM and IgD are expressed on their cell surface and serve as B-lymphocyte antigen receptors. Through these surface antibodies, B lymphocytes detect epitopes on intact antigens *without* the requirement of antigen processing by APCs. After appropriate stimulation of the B-lymphocyte antigen receptor, helper T lymphocyte–B lymphocyte interaction occurs, leading to further B-lymphocyte activation and differentiation. B lymphocytes acquire new functional properties, such as cell division, cell surface expression of accessory molecules, and synthesis of large quantities of antibody. The terminal form of B-lymphocyte differentiation is the plasma cell, which secretes antibodies, or immunoglobulins. Activated B lymphocytes acquire the ability to switch their surface IgM antibodies to another immunoglobulin class (eg, IgG, IgA, or IgE). This class shift requires a molecular change in the immunoglobulin heavy chain and is regulated by specific cytokines released by the helper T lymphocyte. For example, the cytokine IFN- γ induces a switch from IgM to IgG1 production in an antigen-primed B lymphocyte, whereas treatment with IL-4 induces a switch from IgM to IgE.

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Effector Phase

The elimination or neutralization of foreign antigen, which is the purpose of the adaptive immune response, is accomplished during the effector phase. Antigen-specific effectors exist in 2 major subsets (Fig 2-5):

- T lymphocytes, including delayed hypersensitivity and cytotoxic T lymphocytes
- B lymphocytes and the antibodies produced by their derived plasma cells

A third subset of effector lymphocytes—grouped as non-T, non-B lymphocytes—includes natural killer cells, lymphokine-activated cells, and killer cells (see Fig 2-3).

Effector lymphocytes require 2 exposures to antigen for maximum effectiveness. The initial (*priming* or *activation*) exposure occurs in the lymph node. The second (*restimulation*) exposure occurs in the peripheral tissue from which the antigen originated.

Delayed hypersensitivity T lymphocytes usually express the cell marker CD4 and release IFN- γ and TNF- β . Cytotoxic T lymphocytes express CD8 and kill tumor cells and virus-infected host cells. See the section Lymphocyte-Mediated Effector Responses for further discussion of these cells.

The B-lymphocyte effector response is mediated by antibodies produced by plasma cells. Antibodies are released into the efferent lymph fluid (downstream from lymph nodes), draining into the venous circulation. Once bound to their specific epitope, antibodies

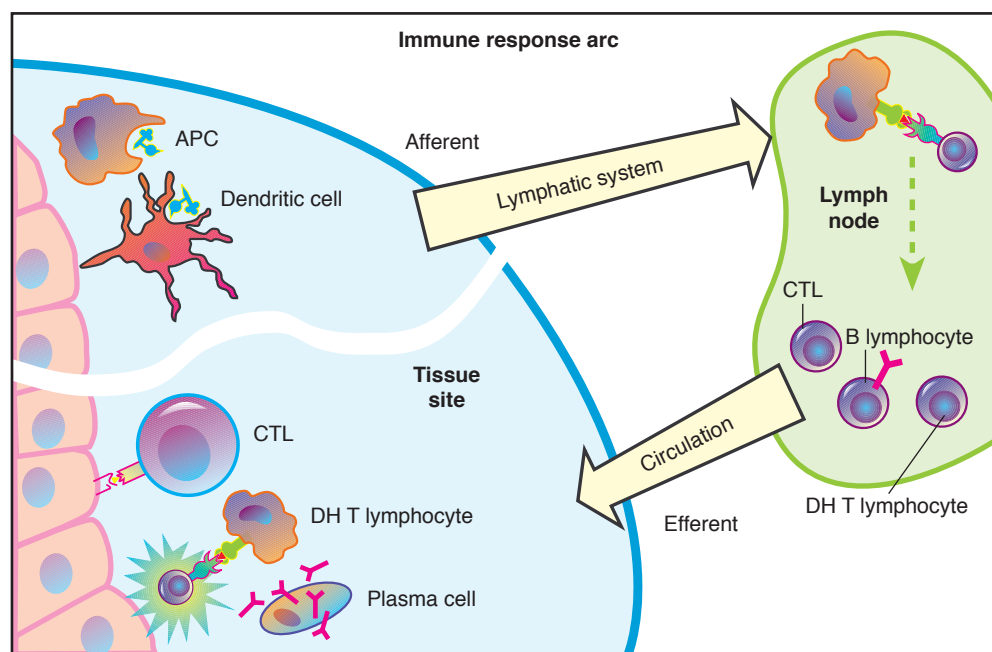


Figure 2-5 Schematic representation of effector mechanisms during the adaptive immune response. The tissue site (*left*) is not only where the immune response is initiated but is also ultimately where the immune response arc is completed—when effectors encounter antigen within tissue after their release from the lymph node (*right*) into the circulation. The 3 most important effector mechanisms of adaptive immunity are CTLs, delayed hypersensitivity (DH) T lymphocytes (CD4⁺ T lymphocytes), and antibody-producing plasma cells derived from B lymphocytes. (Illustration by Barb Cousins, modified by Joyce Zavarro.)

mediate a variety of effector activities, including opsonization for phagocytosis and complement activation via the classical pathway.

The Immune Response Arc and Primary or Secondary Immune Response

Immunologic memory is the most distinctive feature of adaptive immunity. Protective immunization is the prototypical example of this powerful phenomenon.

Differences Between Primary and Secondary Responses

The immune response that occurs on the second or subsequent encounter with an antigen (*anamnestic response*) is regulated differently from that occurring on the first encounter. During the processing phase of the primary response, relatively rare antigen-specific B lymphocytes (perhaps 1 in 100,000 B lymphocytes) and T lymphocytes (perhaps 1 in 10,000 T lymphocytes) must come in contact with appropriately presented antigen. Stimulation of these cells from a completely resting and naive state then occurs, a process that requires days. Following the primary response, various events occur that set the stage for a subsequent rapid and robust secondary response:

- Following stimulation, lymphocytes divide, dramatically increasing the population of antigen-responsive T and B lymphocytes (*clonal expansion*), and migrate to other sites of potential encounter with antigen.
- Upon removal of antigen, T and B lymphocytes activated during the primary response gradually return to a resting state but are no longer naive. They retain the capacity to become reactivated within 12–24 hours of antigen exposure and thus are termed *memory cells*.
- Memory lymphocytes express higher levels of certain cell adhesion molecules, such as integrins, than do naive lymphocytes. Expression of these cell adhesion molecules facilitates *homing*, or migration into target tissues.
- IgM produced during the primary response may be too large to leak passively into a peripheral site. Following antibody-class switching, IgG or other isotypes can leak passively into or be produced at the site; thus, they can immediately bind to antigen and trigger a rapid secondary response.
- In some cases, such as in mycobacterial infection, low doses of antigen may remain in the node or site, producing a chronic, low-level antigenic stimulation of T and B lymphocytes. See Clinical Example 2-1.

CLINICAL EXAMPLE 2-1

Primary and Secondary Response to Tuberculosis

The afferent phase of the primary response begins when alveolar macrophages ingest *Mycobacterium tuberculosis* within the lung and then transport the organisms to the hilar lymph nodes. As T and B lymphocytes

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are primed, the hilar nodes become enlarged. The effector phase begins when the primed T lymphocytes recirculate and enter the infected lung. The T lymphocytes interact with macrophage-ingested bacteria, and cytokines are released that activate neighboring macrophages to differentiate into epithelioid cells, which fuse to form giant cells, eventually forming caseating granulomas. Meanwhile, some effector T lymphocytes become inactive memory T lymphocytes.

A secondary response in the skin is the basis of the tuberculin skin test used to diagnose tuberculosis (TB). The afferent phase of the secondary response begins when a purified protein derivative (PPD) reagent (antigens purified from nontuberculous mycobacteria) is taken up by dermal macrophages after injection. The secondary processing phase begins when the PPD-stimulated macrophages migrate into the draining lymph node, where they encounter memory T lymphocytes, which then reactivate.

The secondary effector phase commences when the reactivated memory T lymphocytes recirculate and home to the dermis, where they encounter additional antigen and macrophages at the injection site, causing the T lymphocytes to become fully activated and release cytokines. Within 24–72 hours, the cytokines induce infiltration of additional lymphocytes and monocytes as well as fibrin clotting. This process produces the typical indurated dermal lesion of the TB skin test, called the *tuberculin form* of delayed hypersensitivity.

Effector Responses and Mechanisms of Adaptive Immunity

In 1962, Gell and Coombs elaborated on 4 mechanisms of adaptive immune-triggered inflammatory responses, creating a classification of allergic reactions comprising types I through IV:

- anaphylaxis
- antibody-dependent cellular cytotoxicity
- immune complex-mediated reactions
- cell-mediated reactions

Familiarity with Gell and Coombs' classifications is important for understanding older literature. However, it is more accurate to divide the effector responses of adaptive immunity into 3 main categories:

- antibody-mediated effector responses
- lymphocyte-mediated effector responses (delayed hypersensitivity, cytotoxic lymphocytes)
- combined antibody and cellular mechanisms

Delves PJ, Martin SJ, Burton DR, Roitt IM. *Roitt's Essential Immunology*. 13th ed. Wiley-Blackwell; 2017.

Owen JA, Punt J, Stranford SA. *Kuby Immunology*. 7th ed. WH Freeman; 2013.

Antibody-Mediated Effector Responses

Structural and functional properties of antibody molecules

Structural features of immunoglobulins There are 5 major classes or isotypes of immunoglobulins (M, G, A, E, and D). IgG and IgA can be further divided into subclasses (IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2). The basic immunoglobulin structure, referred to as a *monomer*, is composed of 4 covalently bonded glycoprotein chains (Fig 2-6):

- 2 identical light chains, either kappa (κ) or lambda (λ)
- 2 identical heavy chains

Each monomer is approximately 150,000–180,000 Da. The type of heavy chain defines the specific immunoglobulin isotype or subclass. IgM can form pentamers or hexamers in vivo, and IgA can form dimers in secretions, so the in vivo molecular size of these 2 classes is much larger than that of the others.

Antibodies contain regions called *domains* that carry out the specific functions of the antibody molecule. The Fab region (2 of which are present on each molecule) contains the antigen-binding domain, called the *variable region*. The opposite end of the molecule, on the heavy chain portion, contains the attachment site for effector cells (the *Fc* [fragment crystallizable] *portion*). It also contains the site of other effector functions, such as complement fixation (for IgG3) or binding to a secretory component for transportation through epithelia and secretion into tears (for IgA). Table 2-1 summarizes key differences between immunoglobulin isotypes.

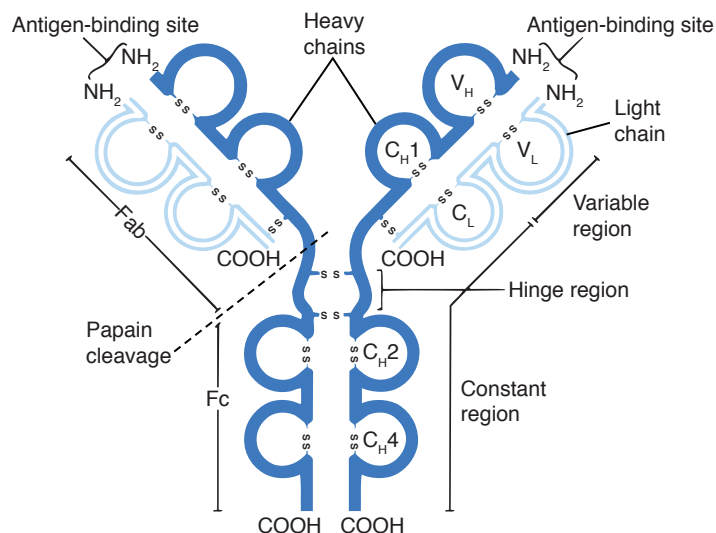


Figure 2-6 Schematic representation of an immunoglobulin molecule. The *solid lines* indicate the 2 identical heavy chains; the *light-blue double lines* indicate the identical light chains; *-ss-* indicates intrachain and interchain covalent disulfide bonds. Fab = fragment, antigen-binding region; Fc = fragment crystallizable region (attachment site for effector cells). (Reproduced with permission from Dorland's Illustrated Medical Dictionary, 32nd ed. Elsevier/Saunders; 2012:921.)

Table 2-1 Structural and Functional Properties of Immunoglobulin Isotypes

Immunoglobulin (Ig) Isotype	Percentage of Total Serum Immunoglobulins	Structural Features	Functions
IgD	<1%	Mostly on surface of B lymphocytes	B-lymphocyte antigen receptor
IgM	5%	Mostly on surface of B lymphocytes or intravascular	B-lymphocyte antigen receptor, agglutination, neutralization, intravascular cytotoxicity, classical complement pathway activation
IgG	77%	Intravascular, in tissues; crosses placenta	Cytotoxicity (IgG1, IgG3), ADCC (IgG2, IgG3), agglutination (IgG3), neutralization (IgG4), classical complement pathway activation (IgG1, IgG3)
IgE	<1%	Mostly in skin or mucosa; bound to mast cells	Mast cell degranulation
IgA	18%	In mucosal secretions, binds secretory component in subepithelial tissues for transepithelial transport and protection from proteolysis	Mucosal immunity, neutralization, alternative complement pathway activation

ADCC = antibody-dependent cellular cytotoxicity.

Functional properties of immunoglobulins Immunoglobulin isotypes differ in how they mediate antibody effector functions. Human IgM and IgG3 are good complement activators, whereas IgG4 is not. Only IgA can bind the secretory component and be actively passed into mucosal secretions. The importance of these differences is that 2 antibodies with identical capacity to bind to an antigen—but of different isotype—will produce different effector and inflammatory outcomes.

Terminology

Clonality Each B cell creates a unique Fab fragment that recognizes a single antigenic configuration. Clonal expansion, or proliferation of an individual B lymphocyte via cell division, results in a population of identical B cells that recognize the same epitope, termed a *monoclonal* population. Biologic drugs such as infliximab, adalimumab, and rituximab are examples of recombinant monoclonal antibodies that specifically target immune system molecules (see Chapter 6). However, the typical immune response is a *polyclonal response* since many individual B cells will recognize various epitopes on the same substance, and each B cell will undergo simultaneous clonal expansion.

Idiotypes Because they are proteins, antibodies themselves can be antigenic. Their antigenic sites are called *idiotopes*, as distinguished from *epitopes*, the antigenic sites on foreign molecules. Anti-idiotypic antibodies may function as feedback mechanisms for immune regulation and have clinical significance for modern biologic therapies. Infliximab, for example, is a monoclonal chimeric (mouse and human) antibody to TNF- α that is used to treat some forms of uveitis. Efficacy of this drug may be limited by the development of anti-idiotypic antibodies that neutralize the antigen-binding site for TNF- α .

Infiltration of B lymphocytes into tissues and local production of antibody

B-lymphocyte infiltration B lymphocytes can infiltrate the site of an immunologic reaction in response to persistent antigenic stimulus. If the process becomes chronic, plasma cell formation occurs, with local production of antibody specific for the inciting antigen(s). Assessment of local antibody production can serve as a diagnostic test (see Clinical Example 2-2) when the antigen is known or suspected, as in the case of a presumed infection.

CLINICAL EXAMPLE 2-2

The Clinical Significance of Local Antibody Production

Distinguishing between local production of antibody and passive leakage of antibody from the blood to the intraocular compartment involves determination of the *Goldmann-Witmer (GW) coefficient*. This is calculated by comparing the ratio of specific IgG antibody present in intraocular fluid to the total IgG level in intraocular fluid versus the ratio of specific IgG level in serum to the total IgG level in serum:

$$\text{GW Coefficient} = \frac{(\text{Intraocular Specific IgG} / \text{Intraocular Total IgG})}{(\text{Serum Specific IgG} / \text{Serum Total IgG})}$$

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Quentin and Reiber used the GW coefficient to demonstrate that aqueous from eyes with Fuchs uveitis syndrome had markedly elevated intraocular IgG titers to rubella virus compared with levels found in controls. The average GW coefficient was 20.6 in patients with Fuchs uveitis syndrome, compared with less than 1.5 in controls.

Quentin CD, Reiber H. Fuchs heterochromic cyclitis: rubella virus antibodies and genome in aqueous humor. *Am J Ophthalmol.* 2004;138(1):46–54.

Local antibody production within a tissue and chronic inflammation Persistence of antigen within a site, coupled with infiltration of specific B lymphocytes and local antibody formation, can produce a chronic inflammatory reaction called the *chronic Arthus reaction*. The histologic pattern often demonstrates lymphocytic infiltration, plasma cell infiltration, and granulomatous features. This type of chronic inflammation may contribute to the pathophysiology of certain chronic autoimmune disorders, such as rheumatoid arthritis, which feature formation of pathogenic antibodies.

Abbas AK, Lichtman AH, Pillai S. *Cellular and Molecular Immunology*. 9th ed. Elsevier/Saunders; 2018.

Mousa HM, Saban DR, Foster CS, Cordero-Coma M, Streilein JW. Allergy and immune-mediated tissue injury. In: Albert DM, Miller JW, Azar DT, Young LH, eds. *Albert and Jakobiec's Principles and Practice of Ophthalmology*. 4th ed. Springer; 2022:837–855.

Lymphocyte-Mediated Effector Responses

Delayed hypersensitivity T lymphocytes

Delayed hypersensitivity (DH) represents the prototypical adaptive immune mechanism for lymphocyte-triggered inflammation. It is especially powerful in secondary immune responses. Previously primed DH CD4⁺ T lymphocytes leave the lymph node, home to local tissues where antigen persists, and become activated by restimulation with the specific priming antigen and HLA class II-expressing APCs. Fully activated DH T lymphocytes secrete mediators and cytokines (see Fig 2-4), leading to the recruitment and activation of macrophages and/or other nonspecific leukocytes. The term *delayed* for this type of hypersensitivity refers to the fact that the reaction becomes maximal 12–48 hours after antigen exposure.

Just as helper T lymphocytes can be divided into 3 subsets—Th1, Th2, and Th17—according to the spectrum of cytokines they secrete, DH T lymphocytes can also be grouped by the same criteria. Experimentally, Th1 cytokines, especially IFN- γ (also known as *macrophage-activating factor*) and TNF- α , activate macrophages to secrete inflammatory mediators and kill pathogens, thus amplifying inflammation. Th1-mediated DH effector mechanisms, therefore, are thought to produce the following effects:

- the classic DH reaction (eg, the PPD skin reaction)
- immune responses to intracellular infections (eg, to mycobacteria or *Pneumocystis* organisms)

Table 2-2 Ocular Inflammatory Diseases Likely Involving Th1-Mediated Delayed Hypersensitivity Effector Mechanisms

Site	Disease	Presumed Antigen
Conjunctiva	Contact hypersensitivity to contact lens solutions	Thimerosal or other chemicals
	Giant papillary conjunctivitis	Unknown
	Phlyctenulosis	Bacterial antigens
Cornea and sclera	Chronic allograft rejection	Histocompatibility antigens
	Marginal infiltrates of blepharitis	Bacterial antigens
	Disciform keratitis after viral infection	Viral antigens
Anterior uvea	Acute anterior uveitis	Uveal autoantigens, bacterial antigens
	Sarcoidosis-associated uveitis	Unknown
	Idiopathic intermediate uveitis	Unknown
Retina and choroid	Sympathetic ophthalmia	Uveal or retinal autoantigens
	Vogt-Koyanagi-Harada syndrome	Uveal or retinal autoantigens
	Birdshot chorioretinopathy	Retinal or uveal autoantigens
Orbit	Acute thyroid orbitopathy	Probably thyrotropin receptor
	Giant cell arteritis	Unknown

Th = T helper.

- immune responses to fungal infections
- most forms of severe T-lymphocyte-mediated autoimmune diseases
- chronic transplant rejection

Table 2-2 summarizes the ocular inflammatory diseases thought to require a major contribution of Th1-mediated DH effector mechanisms.

The Th2 subset of DH cells secretes IL-4, IL-5, and other cytokines. IL-4 can induce B lymphocytes to synthesize IgE, and IL-5 can recruit and activate eosinophils within a site. IL-4 can also induce granuloma formation in response to parasite-derived antigens. Thus, Th2-mediated DH mechanisms are thought to play a major role in the following:

- response to parasitic infections
- late-phase responses in allergic reactions
- asthma
- atopic dermatitis or other manifestations of atopic diseases

Persistence of certain infectious agents, especially bacteria within intracellular compartments of APCs and certain extracellular parasites, can cause destructive induration with granuloma formation and giant cells, termed the *granulomatous form of DH*. Also, immune complex deposition and innate immune mechanisms in response to heavy metal or foreign-body reactions can cause granulomatous inflammation, in which the inflammatory cascade (resulting in DH) is triggered in the absence of specific T lymphocytes. Unfortunately, for most clinical entities in which T-lymphocyte responses are suspected, especially autoimmune disorders such as multiple sclerosis or rheumatoid arthritis, the precise immunologic mechanisms remain highly speculative. See Clinical Example 2-3.

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CLINICAL EXAMPLE 2-3

Toxocara granuloma (Th2 DH) *Toxocara canis* is a nematode parasite that infects up to 2% of all children worldwide. *T canis* infection occasionally produces inflammatory vitreoretinal manifestations (see Chapter 12). Humans become infected through ingestion of viable *T canis* eggs, which subsequently mature into larvae within the intestine. Animal models and immunopathogenesis of human nematode infections at other sites suggest that the primary immune response begins in the gut. The primary processing phase produces a strong Th2 response, leading to a primary effector response with production of IgM, IgG, and IgE antibodies, as well as DH T lymphocytes. Through immune evasion, a few larvae may disseminate hematogenously to the choroid or retina and subsequently invade the retina and/or vitreous. There, a Th2-mediated T-lymphocyte effector response against larva antigens releases Th2 cytokines to induce eosinophil and macrophage infiltration, causing the characteristic eosinophilic granuloma seen in the eye. In addition, antilarval B lymphocytes can infiltrate the eye and are induced to secrete various immunoglobulins, especially IgE. Finally, eosinophils, in part by attachment through Fc receptors, can recognize IgE or IgG bound to parasites and release cytotoxic granules containing the antiparasitic cationic protein directly near the larvae, using a mechanism similar to antibody-dependent cellular cytotoxicity.

Yasuda K, Nakanishi K. Host responses to intestinal nematodes. *Int Immunol*. 2018;30(3):93–102. doi:<https://doi.org/10.1093/intimm/dxy002>

Sympathetic ophthalmia (Th1 DH) Sympathetic ophthalmia is a bilateral granulomatous panuveitis that develops following penetrating trauma to an eye (see Chapter 10). This disorder represents one of the few human diseases in which autoimmunity can be directly linked to an initiating event. In most cases, penetrating injury activates the afferent phase of the immune response. The precise pathogenesis of sympathetic ophthalmia is unclear, but a leading hypothesis is that the injury causes (1) a de novo primary immunization to autoantigens—perhaps because externalization of previously sequestered uveal antigens through the wound initiates an immune response in the conjunctiva or extraocular sites—and (2) a change in the immunologic microenvironment of the retina, retinal pigment epithelium, and uvea to allow an immune response within the eye to overcome local suppressor mechanisms.

The inflammatory effector response is generally thought to be dominated by a Th1-mediated DH mechanism generated in response to uveal or retinal antigens, with CD4⁺ T-lymphocyte predominance early in the disease course. Activated macrophages are also numerous in granulomas, and Th1 cytokines have been identified in the vitreous of affected patients. Th1-mediated DH effector mechanisms also are implicated in many other forms of ocular inflammation (see Table 2-2).

Boyd SR, Young S, Lightman S. Immunopathology of the noninfectious posterior and intermediate uveitides. *Surv Ophthalmol*. 2001;46(3):209–233.

Cytotoxic lymphocytes

Cytotoxic T lymphocytes Cytotoxic T lymphocytes (CTLs) are a subset of antigen-specific T lymphocytes bearing the CD8 marker that are especially good at killing tumor cells and virus-infected cells. CTLs can also mediate graft rejection and some types of autoimmunity. In most cases, the ideal antigen for CTLs is an intracellular protein that occurs naturally or is produced because of viral infection. CTLs appear to require assistance from CD4⁺ helper T-cell signals in order to fully differentiate. Also, local CD4⁺ T lymphocytes and other accessory costimulatory molecules on the target cell may be required to achieve maximal killing.

CTLs kill cells in 1 of 2 ways: “assassination” or “suicide” induction (Fig 2-7). *Assassination* refers to CTL-mediated lysis of target cells. A specialized pore-forming protein called *perforin* is released that inserts into cell membranes and causes osmotic lysis of the cell. *Suicide induction* refers to the capability of CTLs to stimulate programmed cell death of target cells, called *apoptosis*, using the CD95 ligand (FasL) to activate the CD95 receptor (Fas) on target cells. Alternatively, CTLs can release cytokines such as TNF to induce apoptosis. CTLs produce low-grade lymphocytic infiltrates within tumors or infected tissues and usually kill without causing significant inflammation.

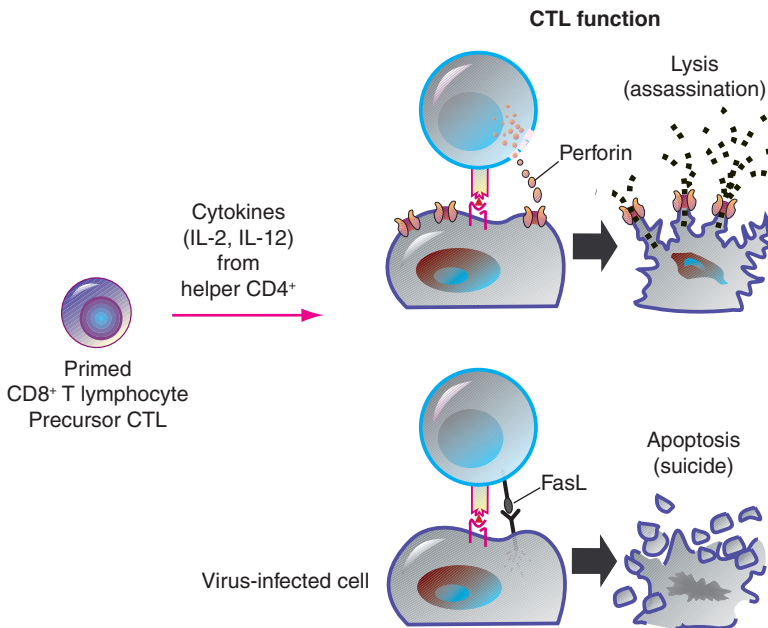


Figure 2-7 Schematic representation of the 2 major mechanisms of CD8⁺ T-lymphocyte cytotoxicity. CD8⁺ T lymphocytes, having undergone initial priming in the lymph node, enter the tissue site, where they again encounter antigen in the form of infected target cells. Upon restimulation, usually requiring CD4⁺ T-cell factors, CD8⁺ T lymphocytes are activated, fully becoming CTLs. CTLs can kill by lysing the infected cell using a pore-forming protein called *perforin* or by inducing programmed cell death, called *apoptosis*, using either Fas ligand (FasL) or cytokine-mediated mechanisms. (Illustration by Barb Cousins, modified by Joyce Zavarro.)

Natural killer cells Natural killer (NK) cells are a subset of non-T, non-B lymphocytes. They kill tumor cells and virus-infected cells using the same molecular mechanisms as CTLs. Unlike CTLs, NK cells do not have a specific antigen receptor and do not require priming or prior activation. Instead, they express multiple activating and inhibitory receptors; the balance of signals from these receptors determines whether NK cells are activated or inhibited. Activating receptors trigger the NK cell to kill target cells that are missing HLA class I molecules or those that display inappropriate molecules. Inhibitory NK receptors prevent NK cells from indiscriminately attacking healthy host tissue by recognizing ligands that ought to be present.

Combined Antibody and Cellular Effector Mechanisms

Antibody-dependent cellular cytotoxicity

An antibody can bind to a cell-associated antigen such as a tumor or viral antigen, but if the antibody is not a member of a subclass that activates complement, the antibody may not directly induce cytotoxicity. However, various leukocytes may recognize the exposed Fc domain of the bound antibody and then activate various leukocyte cytotoxic mechanisms, including degranulation and cytokine production. Classically, *antibody-dependent cellular cytotoxicity* (ADCC) was observed to be mediated by a subset of large granular (non-T, non-B) lymphocytes, called *killer cells*, that induce cell death in a manner similar to CTLs. The killer cell itself is nonspecific but gains antigen specificity through interaction with specific antibody. Macrophages, NK cells, certain T lymphocytes, and neutrophils can also participate in ADCC using other Fc receptor types. An IgE-dependent form of ADCC may also exist for eosinophils.

ADCC is presumed to be important in tumor immune surveillance, antimicrobial host protection, graft rejection, and certain autoimmune diseases, such as cutaneous systemic lupus erythematosus. However, this effector mechanism probably does not play an important role in uveitis, although it might contribute to antiparasitic immunity.

Acute IgE-mediated mast cell degranulation

Mast cells bind IgE antibodies to their surface through a high-affinity Fc receptor specific for IgE molecules, positioning the antigen-recognition site of the bound IgE externally. Combining 2 adjacent IgE antibody molecules with a specific allergen causes degranulation of the mast cell and release of preformed and de novo synthesized mediators within minutes. This acute inflammatory reaction is called *immediate hypersensitivity* (previously called *Gell and Coombs type I*, or *anaphylaxis*).

Preformed mediators include histamine, serotonin, proteoglycans (heparin), neutral proteases (ie, tryptase, chymase), chemotactic factors (eosinophil, neutrophil, or monocyte), and possibly basic fibroblast growth factor. Among the newly generated mediators are the arachidonic acid metabolites prostaglandin D₂, leukotrienes, and thromboxane as well as Th2 cytokines (IL-4, IL-5, IL-13), TNF- α , IL-1, and CCL2.

Releasing these mediators results in vasodilation, increased capillary permeability, contraction of bronchial and gastrointestinal smooth muscle, and increased mucous secretion in mucosal sites. Mast cell-derived cytokines play a role in the late phase of allergic response by activating endothelial cells to recruit eosinophils and other inflammatory cells to the site

of hypersensitivity reactions, thus sustaining inflammation. When severe, the immediate hypersensitivity response can produce a systemic reaction, with manifestations ranging from generalized skin lesions, such as erythema, urticaria, or angioedema, to severely altered vascular permeability with plasma leakage into tissues and airway obstruction or hypotensive shock. The use of antihistamine medications such as diphenhydramine to treat acute allergic reactions illustrates the central role of histamine in immediate hypersensitivity reactions.

Ocular Immune Responses

Highlights

- Unique regional immune responses influence ocular pathology.
- Several immunoregulatory mechanisms modulate the intraocular immune environment as part of the immune privilege of the eye.
- Anterior chamber–associated immune deviation is a recognized mechanism of ocular immune privilege.
- Increased understanding of the immune responses of the eye has been valuable to advances in corneal transplantation, retinal gene therapy, and various developing cell-based therapies.

Regional Immunity and Immunologic Microenvironments

Regional immunity is the concept that many organ and tissue sites demonstrate modifications to the classic immune response arc that may affect some or all 3 phases—afferent, processing, and effector. Tissue-specific or organ-specific variation in immune response is determined by differences in the immunologic microenvironments. There are multiple microenvironments within and around the eye (Table 3-1).

Immune privilege encompasses anatomical, physiologic, and immunologic adaptations that limit inflammatory damage to vital structures. Operationally, immune-privileged sites include the central nervous system, eye, testis, ovary, pregnant uterus, hair follicle, and adrenal cortex. *Ocular immune privilege* is a unique feature of the ocular microenvironment. First described in the 1940s, the concept was based on the observation that foreign antigens placed in the anterior chamber did not elicit an inflammatory response. Multiple mechanisms contribute to ocular immune privilege, including the following:

- physical barriers (partial blood–ocular and blood–retina barriers, lack of efferent lymphatics)
- inhibitory ocular microenvironment (cell-bound and soluble immunosuppressive factors)
- anterior chamber–associated immune deviation (ACAID; suppression of foreign antigen–specific inflammation in the eye)

Zhou R, Caspi RR. Ocular immune privilege. *F1000 Biol Rep*. 2010;2:3. doi:10.3410/B2-3

Table 3-1 Comparison of Immune Microenvironments in Various Normal Ocular Sites				
Anatomical features	Conjunctiva	Cornea, Sclera	Anterior Chamber, Anterior Uvea, Vitreous	Retina, RPE, Choriocapillaris, Choroid
Resident APCs	Lymphatics, follicles	Lymphatics at limbus, none centrally Macromolecules diffuse through stroma	No well-defined lymphatics, antigen clearance through trabecular meshwork	No lymphatics Blood–retina barrier Permeable uveal circulation
	Dendritic and Langerhans cells, macrophages	Langerhans cells at limbus Rare APCs in central and paracentral cornea No APCs in sclera Epithelium/endothelium can be induced to express HLA class II molecules	Partial blood–uveal barrier Many dendritic cells and macrophages in iris and ciliary body Hyalocytes are macrophage derived	Microglia in the retina Dendritic cells and macrophages in choriocapillaris and choroid RPE expresses TLR and can be induced to express HLA class II molecules
	Possibly follicles	None	None	None
Specialized immune compartments for localized immune processing				
Resident effector cells	Mast cells, T lymphocytes, B lymphocytes, plasma cells, rare neutrophils	Central cornea—none Sclera—none	Rare to no T lymphocytes or B lymphocytes, rare mast cells	Retina—normally no lymphocytes Choroid—mast cells, some lymphocytes
Resident effector molecules	All antibody isotypes, especially IgE, IgG subclasses; IgA in tears Complement and kininogen precursors present	Peripherally—all immunoglobulins (minimal IgM) Centrally—minimal antibody, some complement present Sclera—low antibody concentration, minimal IgM	Kallikrein but not kininogen precursors Some complement present, but less than in blood Minimal immunoglobulins in iris, some IgG in ciliary body and aqueous humor	Retina—minimal to no immunoglobulins Choroid—IgG and IgM
Immunoregulatory systems	Conjunctiva-associated lymphoid tissue	Immune privilege—apoptosis-inducing ligands (Fas ligand, TRAIL, PD-L1), avascularity, lack of central APCs	Immune privilege—ACAIID, immunosuppressive factors in aqueous; Fas ligand, TRAIL	Immune privilege—RPE can secrete immunosuppressive molecules (TGF-β2) Complement regulator expression

ACAIID = anterior chamber–associated immune deviation; APC = antigen-presenting cell; HLA = human leukocyte antigen; Ig = immunoglobulin; PD-L1 = programmed death ligand; RPE = retinal pigment epithelium; TGF = transforming growth factor; TLR = Toll-like receptors, TRAIL = tumor necrosis factor–related apoptosis-inducing ligand.

Immune Responses of the Conjunctiva

Features of the Immunologic Microenvironment

The conjunctiva shares many of the features that are typical of mucosal sites. It is well vascularized and has good lymphatic drainage to preauricular and submandibular nodes. The tissue contains numerous Langerhans cells, other dendritic cells, and macrophages that serve as potential antigen-presenting cells (APCs). Certain ocular surface infections or inflammation results in enlarged conjunctival follicles that contain T lymphocytes, B lymphocytes, and APCs. By analogy with similar sites, such as Peyer patches of the intestine, these follicles are likely sites for localized immune processing of antigens that permeate through the thin overlying epithelium. See Chapter 10, Figures 10-7 and 10-9 for examples of conjunctival nodules in sarcoidosis.

The conjunctiva, especially the substantia propria, is richly populated with potential effector cells, predominantly mast cells. All antibody isotypes are represented, with immunoglobulin (Ig) A as the most abundant type in the tears. Local antibody production presumably occurs as well as passive leakage. Soluble molecules of the innate immune system, especially complement, are also present. The conjunctiva appears to support most adaptive and innate immune effector responses, especially antibody-mediated and lymphocyte-mediated responses, although IgE-mediated mast cell degranulation is one of the most common and important. See also BCSC Section 8, *External Disease and Cornea*, for information on conjunctival immune responses.

Immunoregulatory Systems

The conjunctival substantia propria contains *conjunctiva-associated lymphoid tissue (CALT)* and serves as the main T-lymphocyte inductive site of the *ocular mucosal immune system (OMIS)*, which also includes the lacrimal gland, conjunctival and corneal epithelia, and draining lymph nodes. CALT and OMIS are part of the mucosal immune system, which is anatomically and functionally distinct from the systemic (primarily bloodborne) immune compartment. The mucosal immune system utilizes innate and adaptive immune responses to maintain the homeostasis of mucosal surfaces. The OMIS can be divided into mucosal inductive sites and mucosal effector sites. APCs take up and process antigens (pathogens and allergens) and then present them to T lymphocytes at mucosal inductive sites, usually in local (cervical, preauricular, and submandibular) lymph nodes. Antigen-specific T lymphocytes are induced in these lymph nodes and then return to ocular mucosal effector sites, where they carry out their immune functions.

Within the mucosal immune system, certain sites may elicit a more far-reaching distal mucosal immune response than others. Due to the nasolacrimal drainage system, there is direct anatomical communication between the OMIS and the *nasal-associated lymphoid tissue (NALT)*, which are thought to be immunologically connected and interdependent. For example, intranasal immunization induces IgA within the nose and salivary glands as well as on the ocular surface and may elicit more tear IgA antibody responses than does direct ocular exposure. Increased understanding of the integrated nature of the OMIS and NALT

systems has influenced the development of intranasally administered immunotherapeutic agents against inflammatory dry eye disease.

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Immune Responses of the Cornea

Features of the Immunologic Microenvironment

The cornea is unique among ocular tissues because the peripheral and central portions of the tissue represent distinctly different immunologic microenvironments. In normal eyes, only the limbus is vascularized and richly invested with Langerhans cells. The avascular paracentral and central cornea are mostly devoid of APCs. Various stimuli, such as mild trauma, certain cytokines (eg, interleukin 1), or infection, can recruit APCs to the central cornea. Immune-mediated corneal changes such as marginal keratitis often occur adjacent to the vascularized limbus.

Plasma-derived proteins (eg, complement and IgG) are present in moderate concentrations in the periphery, but only low levels of IgM are present centrally. Corneal cells also synthesize various antimicrobial and immunoregulatory proteins. Localized immune processing probably does not occur in the cornea, and effector cells are scarce or absent; however, neutrophils, monocytes, and lymphocytes can readily migrate through the stroma if appropriate chemotactic stimuli are activated. These immune cells can also adhere to the endothelial surface during inflammation, giving rise to keratic precipitates or the classic Khodadoust line of endothelial rejection (Fig 3-1). See also BCSC Section 8, *External Disease and Cornea*.

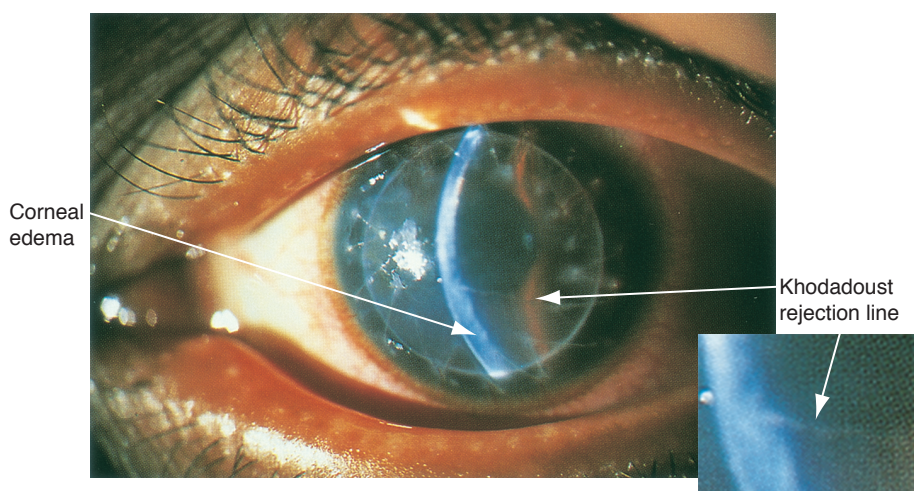


Figure 3-1 Endothelial graft rejection with stromal and epithelial edema on the trailing aspect of the migrating Khodadoust line (*inset*).

Immunoregulatory Systems

Immune privilege of the cornea is multifactorial. Normal limbal physiology is a major component, especially the maintenance of avascularity and the scarcity of APCs in the paracentral and central cornea. The lack of APCs and lymphatic channels partially inhibits the afferent response in the central cornea. The absence of postcapillary venules centrally can limit the efficiency of effector recruitment, although effector cells and molecules can infiltrate even avascular cornea. The corneal endothelium is exposed to anti-inflammatory and immunosuppressive molecules in the aqueous humor such as transforming growth factor β , α -melanocyte-stimulating hormone, macrophage migration inhibitory factor, and vasoactive intestinal peptide. In addition, corneal epithelial and endothelial cells have cell membrane-bound molecules such as Fas ligand (FasL) that resist immunologic attack by inducing apoptosis of infiltrating neutrophils and lymphocytes that express the FasL receptor. (See Clinical Example 3-1.)

CLINICAL EXAMPLE 3-1

Corneal Allograft Rejection

Penetrating keratoplasty, or the transplantation of full-thickness corneal allografts, has an extremely high success rate (>90%) even in the absence of systemic immunomodulation. This rate is substantially superior to acceptance rates after transplantation of other donor tissues. Corneal allograft survival has been attributed to the mechanisms of immune privilege, including suppression of the immune response against allograft antigens, generation of regulatory T (Treg) lymphocytes that can suppress the destructive alloimmune reaction, and induction of apoptosis of inflammatory cells at the graft-host interface. Experimental models and clinical studies show that factors contributing to rejection include the following:

- stimuli for corneal vascularization that also induce ingrowth of lymphatic vessels and infiltration of dendritic cells
- induction of stromal human leukocyte antigen (HLA) molecule expression (normally low)
- contamination of the donor graft with donor-derived APCs prior to the transplant
- loss of immune privilege via maneuvers (eg, splenectomy) that prevent induction of ACAID

Changes in host immune status may influence graft rejection as well. Graft rejection has been reported in association with systemic checkpoint inhibitor treatment, SARS-CoV-2 infection, and recent vaccination; however, isolated case reports are not necessarily evidence of causality.

Niederhorn JY. The eye sees eye to eye with the immune system: the 2019 Proctor Lecture. *Invest Ophthalmol Vis Sci.* 2019;60(13):4489–4495.

Yin J. Advances in corneal graft rejection. *Curr Opin Ophthalmol.* 2021;32(4):331–337.

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Immune Responses of the Anterior Chamber, Anterior Uvea, and Vitreous

Features of the Immunologic Microenvironment

The anterior chamber is a fluid-filled cavity; circulating aqueous humor provides a unique medium for intercellular communication among immune cells and resident tissue cells of the iris, ciliary body, and corneal endothelium. Although aqueous humor is relatively protein depleted compared with serum (containing about 0.1%–1.0% of the total serum protein concentration), even normal aqueous humor contains a complex mixture of biological factors, such as immunomodulatory cytokines, neuropeptides, and complement inhibitors, that influence immunologic events within the eye.

A partial blood–ocular barrier is present in the anterior chamber: fenestrated capillaries in the ciliary body allow a size-dependent concentration gradient of plasma macromolecules to permeate the interstitial tissue. Smaller plasma-derived molecules are present in higher concentration than larger molecules. The tight junctions between the pigmented and nonpigmented ciliary epithelium provide a more exclusive barrier, preventing interstitial macromolecules from permeating directly through the ciliary body into the aqueous humor. Nevertheless, a small number of plasma macromolecules bypass the nonpigmented epithelium barrier and enter the anterior chamber by diffusion directly through the anterior iris surface.

A few resident T lymphocytes and mast cells are present in the normal anterior uvea; B lymphocytes, eosinophils, and neutrophils are usually absent. Very low concentrations of IgG and complement components occur in normal aqueous humor. The iris and ciliary body contain significant numbers of macrophages and dendritic cells that serve as APCs and possible effector cells, but immune processing is unlikely to occur locally. Since the inner eye does not contain well-defined lymphatic channels, clearance of soluble substances depends on aqueous humor outflow channels. Nevertheless, antigen inoculation into the anterior chamber results in efficient communication with the systemic immune system. Particulates can be removed via endocytosis by trabecular meshwork endothelial cells or macrophages. Intact soluble antigens can enter the venous circulation that transports them to the spleen.

The vitreous has not been studied as extensively as the anterior chamber. Studies employing proteomics reveal the vitreous as a physiologically active, complex tissue containing diverse proteins originating from inside and outside the eye. An important source of these inflammatory mediators is the retina. The vitreous gel can electrostatically bind charged protein substances to serve as an antigen depot as well as a substrate for leukocyte cell adhesion. The vitreous may also serve as an autoantigen because of the presence of collagen type II, which has been implicated in the pathogenesis of rheumatoid arthritis. Hyalocytes, which are modified resident macrophages, are important in vitreal immunoregulation/modulation and frequently act as APCs. They also respond to different cytokines, playing a role in the immunopathogenesis of several disorders, including proliferative vitreoretinopathy.

Immunoregulatory Systems

Relatively mild inflammation that would be harmless in the skin can cause severe vision loss in the eye. Fortunately, a variety of immunoregulatory mechanisms modulate

intraocular immune responses, including in the anterior chamber, anterior uvea, and vitreous.

Ocular immune privilege has been observed with various antigens, including alloantigens (eg, transplant antigens), tumor antigens, haptens, soluble proteins, autoantigens, bacteria, and viruses. The best-studied mechanism of immune privilege in the eye is ACAID. Although subcutaneous inoculation of antigen elicits a strong, delayed hypersensitivity (DH) reaction, anterior chamber inoculation with the identical antigen results in a robust antibody response but a virtual absence of DH. In fact, preexisting DH can be suppressed by ACAID.

ACAID represents an attenuated effector arc in which cell-mediated immune responses such as DH and cytotoxic T-lymphocyte responses are suppressed by regulatory T (Treg) cells. ACAID blocks T helper-1 (Th1) immune responses and blunts Th2-mediated inflammatory disease. ACAID also shifts the antibody responses to preferential production of non-complement-fixing antibodies, thus reducing the likelihood of antibody-mediated ocular tissue injury from activation of the complement cascade.

Note that the immunoregulatory environment can still be overcome by sufficient immune stimulation.

Boneva SK, Wolf J, Rosmus DD, et al. Transcriptional profiling uncovers human hyalocytes as a unique innate immune cell population. *Front Immunol.* 2020;11:567274. doi:10.3389/fimmu.2020.567274

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Skeie JM, Roybal CN, Mahajan VB. Proteomic insight into the molecular function of the vitreous. *PLoS One.* 2015;10(5):e0127567.

Immune Responses of the Retina, Retinal Pigment Epithelium, Choriocapillaris, and Choroid

Features of the Immunologic Microenvironment

The immunologic microenvironments of the retina, retinal pigment epithelium (RPE), choriocapillaris, and choroid have not been well characterized. An inner blood–ocular barrier is formed by tight junctions between the endothelial cells of the retinal vasculature, while tight junctions between the cells of the RPE provide an outer barrier between the choroid and the retina. The vessels of the choriocapillaris are highly permeable to macromolecules and allow transudation of most plasma macromolecules into the extravascular spaces of the choroid and choriocapillaris. Well-developed lymphatic channels are absent, although both the retina and the choroid have abundant potential APCs. In the retina, resident microglia (bone marrow–derived cells related to dendritic cells) are interspersed between the ganglion cell and outer plexiform layers and can undergo physical changes and migrate in response to various stimuli. The choriocapillaris and choroid contain an abundance of potential APCs, especially macrophages and dendritic cells.

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The RPE can be induced to express human leukocyte antigen (HLA) class II molecules, suggesting that the RPE may interact with T lymphocytes in some circumstances. The presence of T lymphocytes or B lymphocytes within the normal posterior segment is not well characterized, but effector cells appear to be absent from the normal retina. Local immune processing does not seem to occur, either. Similar to macrophages, RPE cells also express Toll-like receptors. These special pattern recognition receptors are critical in the detection of pathogens and in the initiation of innate and adaptive immune responses, forming an initial line of defense against invading microorganisms.

A moderate density of mast cells is present in the choroid, especially around the arterioles, but lymphocytes are present only in very low numbers. Eosinophils and neutrophils appear to be absent. However, under various clinical or experimental conditions, a large number of T lymphocytes, B lymphocytes, macrophages, and neutrophils can infiltrate the choroid, choriocapillaris, and retina. The choroid can function as a repository for immunoreactive cells, manifesting clinically as lymphoid hyperplasia. It is hypothesized that the concentration of mast cells in the choroid may facilitate the spread of immunoreactive cells to other parts of the eye. The RPE and various cell types within the retina and choroid (eg, pericytes) can synthesize many different cytokines (eg, transforming growth factor β 2) that may alter the subsequent immune response. See also BCSC Section 12, *Retina and Vitreous*.

McMenamin PG, Saban DR, Dando SJ. Immune cells in the retina and choroid: two different tissue environments that require different defenses and surveillance. *Prog Retin Eye Res*. 2019;70:85–98. doi:10.1016/j.preteyeres.2018.12.002

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Immunoregulatory Systems

A mechanism of immune privilege, likely similar to ACAID, is present after subretinal injection of antigen. Iris, ciliary body, and RPE cells all contribute to immune homeostasis that is mediated by soluble or membrane-bound molecules. For example, soluble factors secreted by RPE cells can suppress APC and effector T-lymphocyte activation and induce Treg and myeloid-derived suppressor cell activity. These observations may be important because of growing interest in retinal transplantation, stem cell therapies, and gene therapy. (See Clinical Example 3-2.) The capacity of the choriocapillaris and choroid to

CLINICAL EXAMPLE 3-2

Retinal Gene Therapy

Many current retinal gene therapies are achieved through the transfection of neural retinal cells or RPE with a replication-defective virus that has been genetically altered to carry a replacement gene. This gene becomes expressed in any cell infected by the virus. Immune clearance of the virus can cause loss of expression of the transferred gene in other body sites.

In 2017, the US Food and Drug Administration approved the first gene replacement therapy for biallelic *RPE65* mutation–associated retinal dystrophy (Leber congenital amaurosis). The agent, voretigene neparvovec-rzyl, uses an adeno-associated viral (AAV) vector to replace defective *RPE65*. It is administered via subretinal injection. Studies are currently under way to investigate gene therapy utilizing AAV for various inherited retinal dystrophies, such as achromatopsia, choroideremia, Leber hereditary optic neuropathy, X-linked retinoschisis, and X-linked retinitis pigmentosa. See also BCSC Section 12, *Retina and Vitreous*.

Despite the relative immune privilege of the eye, immunogenicity of viral vectors is an ongoing area of investigation. In animal models, intravitreal viral vector delivery has led to a humoral response, whereas subretinal delivery has not. Intraocular inflammation was observed in approximately 5% of patients receiving voretigene neparvovec-rzyl; therefore, systemic corticosteroids are started 3 days before injection of this agent.

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function as unique environments for the afferent or effector phases of the immune response has not yet been evaluated.

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